University of Alberta

Therapeutic Support for the Systemic and Regional Hemodynamics in the Asphyxiated Newborn Piglet Resuscitated with 21% and 100% Oxygen

By

©

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Dedications

To His Highness Shaiek Zayed bin Sultan Al Nahyan, the late president and the establisher of the United Arab Emirates who believed in women's freedom of choice.

To my parents who taught me the love for knowledge and reading.

To my husband Dhiaa, I am grateful for all the support encouragement, and patience.

Abstract

Neonatal asphyxia is often associated with shock and pulmonary hypertension. Epinephrine and dopamine are commonly used to treat neonates with hypotension. With the current controversy over 21% versus 100% oxygen for newborn resuscitation, the hemodynamic response and the proxidant effect of epinephrine and dopamine following neonatal hypoxia-reoxygenation was examined in randomized blinded fashion. Newborn piglets were acutely instrumented. The low dose of either drug had no effect on systemic or renal perfusion. Compared to dopamine, epinephrine (0.5-2 mcg/kg/min) was a more effective inotrope to treat shock and hypotension in asphyxiated infants resuscitated with 100% oxygen, without compromising the regional flow, but was associated with an increased myocardial oxidative stress. Dopamine (10-25 mcg/kg/min) had a weak vasopressive action and increased mesenteric arterial flow.

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Table of Contents Page

Chapter 1: Neonatal hypoxia and asphyxia--1

1-1. Neonatal hypoxia--- ------2

1-2. Initial physiological response to neonatal hypoxia----------------------------------3

1-3. Systemic and regional hemodynamics changes during hypoxia-------------------4

1-4. Cellular metabolic changes in response to hypoxia----------------------------------6

References---13

Chapter 2: Hypoxia-Reoxygenation injury---17

2-1. Oxidative stress and reactive oxygen production------------------------------------19

2-2. Oxygen is an important component of ROS generation---------------------------20

2-3. Source of ROS during hypoxia-reoxygenation injury ----------------------------22

2-4. Antioxidant mechanisms--26

2-5. Antioxidant mechanisms during neonatal period---------------------------------29

2-6. Cellular damage induced by oxidant stress---30

2-7. Clinical sequelae of oxidant stress in the neonates--------------------------------31

References----- --42

Chapter 3: 21% versus 100% oxygen in neonatal asphyxia-----------------------51

References---59

Chapter 4: Cardiovascular support during neonatal hypoxia-reoxygenation-63

4-1. Control mechanisms in cardiovascular functions---------------------------------64

4-2. Adrenergic and dopamine receptors---67

4-3. Pharmacology of epinephrine and dopamine--------------------------------------74

4-4. Use of epinephrine and dopamine in the treatment of shock in neonates-------79

4-5. Antioxidant and proxidant properties of catecholamines------------------------90

References ---103

Chapter 5: The research project---112

5-1. Introduction--113

5-2. Newborn piglet models in neonatal research-------------------------------------117

References--120

Chapter 6: General objective and hypotheses-----------------------------------125

Chapter 7: The treatment of shock in asphyxiated newborn piglets resuscitated with 21% and 100%: epinephrine vs. dopamine-------------------129

References--158

Chapter 8: The effect of epinephrine and dopamine on the myocardial glutathione in asphyxiated newborn piglets resuscitated with 21% or 100%-162

References--179

Chapter 9: Conclusions and limitations---181

Appendices--186

A1. Novel therapies in neonatal pulmonary hypertension ------------------------187

References--193

A2. Intratracheal administration of sildenafil and surfactant alleviates the pulmonary hypertension in newborn pig---------------------------196

References--214

A3. Definitions--218

List of Tables page

1-1 Causes of neonatal asphyxia 10

1-2 Complication and system involvement in severe hypoxia 11

3-1 Summary of the clinical studies of 21% oxygen versus 100% oxygen in neonatal resuscitation 55

3-2 Summary of the clinical studies of 21% oxygen versus 100% oxygen in neonatal resuscitation 57

4-1 Adrenergic receptor classification, location and function 93

4-2 Tissue distribution of β adrenergic receptors 94

4-3 Dopamine receptors 95

4-4 Cardiovascular effects of epinephrine at various infusion rates 96

4-5 Cardiovascular effects of dopamine at various infusion rates 97

4-6 Pharmacokinetics of epinephrine and dopamine 98

4-7 Afferent inputs in cardiovascular control 99

7-1 Baseline hemodynamic parameters for the experimental groups 147

7-2 Hemodynamic parameters for the experimental groups at the end of hypoxia. 148

7-3 Hemodynamic parameters at end of 4.5 h of reoxygenation 149

7-4 Hemodynamic changes during low and optimal doses of epinephrine and dopamine 150

7-5 Regional flow index at low and optimal doses of epinephrine and dopamine compared to control 151

7-6 Systemic oxygen delivery, consumption and extraction at low and optimal doses of epinephrine and dopamine compared to respective controls 152

7-7 Regional oxygen delivery at low and optimal doses of epinephrine and dopamine compared to control 153

8-1 Hemodynamic variables at baseline (epinephrine proxidant effect) 174

8-2 Cardiac Left ventricle glutathione components levels 175

A2-1 Physiological characteristics (inhaled sildenafil) 209

A2-2 Systemic oxygen metabolism (inhaled sildenafil) (inhaled sildenafil) 210

List of Figures Page

1-1 Glucose metabolism and energy production 12

2-1 Oxygen reduction reactions 37

2-2 The formation of reactive oxygen species 38

2-3 The formation and detoxification of peroxynitrite 39

2-4 Hypoxanthine-xanthine free radical generation 40

2-5 Reduction of hydrogen peroxide 41

4-1 Adrenoreceptors activation and signal transduction mechanism 100

4-2 Epinephrine and dopamine synthesis and metabolism 101

4-3 The chemical structures of epinephrine and dopamine 102

7-1 Systemic arterial pressure response to epinephrine and dopamine 154

7-2 Cardiac index response to epinephrine and dopamine 155

7-3 Pulmonary to systemic arterial pressure ratio response to epinephrine and dopamine 156

7-4 Pulmonary arterial pressure response to epinephrine and dopamine 157

8-1 Cardiac left ventricle GSSG in the sham and groups received 100% oxygen 177

8-2 Cardiac left ventricle GSSG/TG in the sham and groups received 100%oxygen 178

A2-1 Pulmonary vascular resistance response to low dose sildenafil 212

A2-3 Pulmonary to systemic arterial pressure ratio response to low dose sildenafil 213

List of Abbreviations

α-AR – alpha adrenoreceptor

β-AR – Beta adrenoreceotors

AC- Adenylyl cylase

ADP- Adenosine diphosphate

AMPA – Amino-3-hydroxy-5-methyl-4-isoxazole propionate

ANOVA - Analysis of varaince

AR - Adrenoreceptors

ATP- Adenosine 5-triphosphate

cAMP- Cyclic adenosine monophosphate

CI cardiac index

cGMP- cyclic guanine ribonucleotide

COMT – Catechol-o-methyltransferase

CoA- coenzyme A

CSF – cerebrospinal fluid

DG-Diacylglycerole

DNA - Deoxyribose nucleic acid

DOPAC – 3,4-Dihydroxyphenylacetic acid

DR-Dopamine receptors

EC-SOD – Extracellular superoxide dismutase

EDCF – Endothelium contracting factor

PTH- Pulmonary hypertension

PVRI- Pulmonary vascular resistance index

GABA - Gamma-aminobutyric acid

GC- Guanylate cyclase

GDP- Guanosine driphosphate

GSH - Reduced glutathione

GSSG - oxidized glutathione

GR - Glutathione reductase

GTP- Guanosine triphosphate

Kv - Voltage-dependent potassium channels

HIE - Hypoxic ischemic encephalopathy

$HO^{\bullet}$ - Hydroxyl radical

H_2O_2 - Hydrogen peroxide radical

HOCL- Hypohalous acids

HVA- Homovanillic acid

IP3- Triphosphate

I/R- Ischemia reprfusion

L-arg- L-arginine

LCCAI- Left common carotid artery index

EDHF - Endothelium hyperpolarizing factor

EDRF - Endothelium-derived relaxing factor

FAD- Flavin adenine dinucleotide

$FADH_2$ – Reduced FAD

Fisher LSD – Fisher least significant difference

NAMDA - N-methyl-D-aspartate

NEC- Necrotizing enterocolitis

NNT- Number needed to treat

NICU-Neonatal intensive care unit

NO- Nitric oxide

$NO^{\bullet}$ - Nitric oxide radical

NOS - Nitric oxide synthase

$O_2^{\bullet-}$ - Superoxide radical

$ONOO^-$ - Peroxynitrite

ONOOH - Peroxynitrous acid

$PaCO_2$ - Arterial blood carbon dioxide partial pressure

PaO_2 - arterial blood oxygen partial pressures

PAP- Mean Pulmonary Arterial Pressure

PC- Pyruvate carboxylase

PDH- pyruvate dehydrogenase

PLC- Phospholipase C

LRAI- Left renal artery index

MAO - Monoamine oxidase

MHPC- 3-methoxy-4-hydroxyphenylglycol

NAD- Nicotinamide adenine dinucleotide

NADH - Nicotinamide adenine dinucleotide phosphate

$ROO^{\cdot}$ - Peroxyl radical

ROS - Reactive oxygen species

SAP - Systemic blood pressure

SMAI - Superior mesenteric artery index

SOD - Superoxide Dismutase

SVI - Stroke volume index

SVRI - Systemic vascular resistance index

$SysDO_2$ - Systemic oxygen delivery

$SysEO_2$ - Systemic oxygen extraction

$SysVO_2$ - Systemic oxygen consumption

TCA - Tricyclic acid cycle

XD - Xanthine dehydrogenase

XO - Xanthine oxidase

VMA - Vanillylmandelic acid

Chapter 1

Neonatal hypoxia and asphyxia:

Hemodynamic and cellular responses

1-1. Neonatal asphyxia

Asphyxia is a severe form of hypoxia associated with cardiovascular decompensation. Perinatal asphyxia is asphyxia relating to the period around childbirth and may result in neonatal asphyxia.[1] Neonatal asphyxia may follow severe hypoxia in early postnatal life (Table1-1). In addition to the clinical presentation of shock and hypotension, asphyxia is defined biochemically as hypoxemia, hypercapnia, and mixed acidosis in combination.[2] In order to examine the effects of severe hypoxia and reoxygenation, the definition of asphyxia as severe hypoxia with acidosis and clinical cardiovascular decompensation is used in this thesis.

In the neonatal period asphyxia can be a result of neonatal and fetal hypoxia which can be attributed to maternal and fetal factors (Table1-1). When oxygen deficiency occurs, physiological compensations provide some defense against hypoxia so that the effects are reduced, but prolonged exposure to hypoxia or severe oxygen deficiency can result in multi-organ dysfunction (Table1-2) and serious consequences.[3,4]

1-2. Initial physiological response to neonatal hypoxia

The fetus or newborn that is subjected to hypoxia begins a "diving" reflex (so termed because of certain similarities to the physiology of diving seals) in an attempt to maintain perfusion and oxygen delivery to vital organs. Pulmonary vascular resistance increases, leading to a decreased pulmonary blood flow and increased blood flow directly to the left atrium. Systemic cardiac output is redistributed, with increased flow to the heart, brain and adrenal glands and decreased flow to the rest of the body.[5] The newborn heart may have limited ability to increase its contractile state when subjected to an insult, so that the heart rate usually increases to improve hemodynamic status.[6] Early in hypoxia, systemic blood pressure may increase. However, with ongoing hypoxia and acidosis the myocardium fails and systemic vasodilation develops, so that the blood pressure begins to decrease leading to tissue ischemia and hypoxia.

The respiratory pattern may also be altered by hypoxia. Initially there are rapid respirations, but these respiratory efforts eventually cease with continued hypoxia (termed primary apnea). During primary apnea, the infant responds to stimulation with reinstitution of breathing. However, if the hypoxia continues, the infant then begins irregular gasping efforts, which slowly decrease in frequency and eventually cease altogether (termed secondary apnea). Infants who experience secondary apnea do not respond to stimulation and require positive pressure ventilation to restore respiration.[7]

1-3. Systemic and regional hemodynamic changes during hypoxia

The reported systemic and regional responses to hypoxia have been variable. These variations may be due to several factors: (1) degree and duration of hypoxemia used,[8] (2) size of the blood vessel,[9] (3) difference in species,[10,11,12] (4) use of anesthesia,[13] and (5) type of cells affected. Different parenchymal cells have different thresholds for ischemia and hypoxia: neurons (3-4 min), cardiac muscle, hepatocytes, renal tubular cells and gastrointestinal epithelium (20-80 min), fibroblasts, epidermis and skeletal muscle (hours).[14]

Cheung et al studied the temporal effect of hypoxia on the systemic and regional circulation in newborn piglets.[15] Animals were exposed to severe hypoxemia (defined as arterial saturation 40-50%) for 3 h. During severe hypoxia the cardiac output and stroke volume increased transiently for 30-60 min. The cardiac output returned to baseline value at 3 h of hypoxia. The unsustained increase in cardiac output can be explained by the progressive myocardial failure induced by acidosis and hyperlactatemia produced when the myocardial cell switched to anaerobic glycolysis to maintain ATP supply.[16,17] During early hypoxia, a significant drop in systemic vascular resistance occurs. This systemic vasodilation in response to hypoxia increases perfusion to the hypoxemic tissue and it is mediated by vasodilator metabolites such as adenosine[18] and increased production of endothelial nitric oxide (NO) and carbon monoxide.[19,20] Like NO, carbon monoxide is an endogenously produced gas molecule. There are at least two endogenous sources of carbon monoxide production, one which is from the oxidation of organic molecules, but the predominant source is from the degradation of heme by heme oxygenase.[21] Carbon monoxide and NO both mediate the vasodilation effect through the activation of guanylyl cyclase to increase levels of 3'5'-cyclic guanosine monophosphate (cGMP).[20] Carbon monoxide can also acts via a non-cGMP dependent mechanism. Both cGMP-dependent and non-cGMP dependent vasodilation may be important for vascular tone regulation during hypoxia in fetus and neonates.[22]

The pulmonary pressure and vascular resistance increase during hypoxia. The pulmonary hypoxic response is induced by several mechanisms; increased production of vasoactive agents such as endothelin,[23] decreased production or the inhibition of the vasodilator NO,[24] and the activation of calcium and potassium channels.[25]

The mesenteric hypoxic response shows a biphasic pattern with initial vasoconstriction, followed by vasorelaxation to baseline as hypoxia continues.[15,26] The mechanism of the biphasic response of the mesenteric circulation could be explained by an initial sympathetic mediated vasoconstriction, which is then reversed by the accumulation of local vasorelaxant metabolites.[27]

The kidney is one of the commonly damaged organs in asphyxiated full-term infants[28] and it has been extensively studied. Hypoxia induces a significant reduction in renal blood flow, glomerular filtration rate and an increase in renal vascular resistance.[29]

The microvascular blood perfusion in the cortex was studied by Kutzsche et al, who showed that the regional cortical microvascular perfusion increased significantly after 5 min of hypoxia by 26-49%. This increase remained at a steady state during the period of hypoxia for (around 1 h) and was approximately 39-49% higher than baseline.[30]

1-4. Cellular metabolic changes in response to hypoxia

Under normoxia, oxygen is utilized in oxidative phosphorylation to provide the cell with high energy adenosine 5-triphosphate (ATP), the basic energy fuel needed for the cellular functions (Fig1-1). Fuel molecules mostly glucose are oxidized to generate ATP and reducing agents like nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FDAH_2$). NADH is produced during the glycolysis reaction [1].

Glucose + 2 HPO_4^{2-} + 2 ADP + 2 NAD^+ $\rightarrow$ 2 $Pyruvate^-$ + 2 ATP^- + 2 NADH + 2 H^+ + 2 H_2O [1]

The fate of pyruvate in the mitochondria depends on the cell energy charge; when transported into the mitochondrion, pyruvate encounters two principal metabolizing enzymes: pyruvate carboxylase, a gluconeogenic enzyme, and pyruvate dehydrogenase. In cells or tissues with a high energy charge pyruvate is directed toward gluconeogenesis, when the energy charge is low, pyruvate is preferentially oxidized to CO_2 and H_2O in the tricarboxylic acid cycle as acetyl CoA [2] [3].

2 Pyruvate + 2 Coenzyme A +2 NAD^+ $\rightarrow$2 Acetyl CoA +2 CO_2 +2 NADH [2]

2 Acetyl CoA + 6 NAD^++2 FAD + 2 GDP +4 HPO_4^{2-} + 4 H_2O $\rightarrow$ 4 CO_2 + 6 NADH + 2 $FADH_2$ +2 GTP +4 H^+ + 2 Coenzyme A [3]

The processing of 1 molecule of glucose through glycolysis and tricarboxylic acid cycle reaction generates around 11% of aerobic ATP production. The NADH and $FDAH_2$ generated in these reactions will then carry the hydrogen ions (absorbed energy) to the mitochondria for oxidative phosphorylation. In oxidative phosphorylation NADH and $FDAH_2$ are oxidized to release the hydrogen ions which are taken by oxygen in number of steps to produce water and ATP (88% of aerobic ATP production). The oxidized NAD and FDA will then return to the tricarboxylic acid cycle for recycling.

Hypoxia is known to modulate a number of cellular metabolic functions; under anaerobic conditions, pyruvate is converted to lactate by the enzyme lactate dehydrogenase, thus anaerobic glycolysis generates only 6% of aerobic ATP production. Lactate is transported out of the cell into the circulation. The conversion of pyruvate to lactate, under anaerobic conditions, provides the cell with a mechanism for the oxidation of NADH to NAD^+ without which glycolysis will cease.

The depletion of cellular energy supply has a deleterious effect on the cellular functions. Following the cessation of energy-dependent functions such as contractile function in myocytes, the plasma membrane ion pump mechanisms gradually become dysfunctional and the cell become incapable of maintaining transmembrane ionic gradients. The failure of sodium and potassium ATPase will result in efflux of potassium and influx of sodium and cell depolarization. Cell depolarization opens voltage-dependent calcium channels and increases in cellular calcium concentration. The higher intracellular calcium concentration activates membrane phospholipases which catalyze hydrolysis of cell membrane, organelle and mitochondrial phospholipids. This cellular homeostatic disruption can lead to cell swelling, lysis and death.

The other important effect of oxygen depletion is the initiation of oxidative stress. As mentioned above, normally, most of the oxygen in the tissue is used in oxidative metabolism by the cytochrome system in the mitochondria, where four electrons are donated to O_2 (tetravalent reduction) in a single process. Over 95% of oxygen undergoes tetravalent reduction to produce water and energy in the form of ATP.[31] Only a small proportion of oxygen normally is reduced by a sequential and divalent pathway in the mitochondria and other electron transport process in the nuclear membrane and the endoplasmic reticulum. This partial reduction of oxygen leads to the formation of reactive oxygen species (ROS).[32] Univalent reduction of oxygen is catalyzed by xanthine oxidase (XO) to produce superoxide radical ($O_2^{\bullet-}$) [4], while divalent reduction of oxygen results in the formation of hydrogen peroxide (H_2O_2) [5].

$$O_2 + e^- \xrightarrow{XO} O_2^{\bullet-} \quad [4]$$

$$O_2 + 2\,H^+ + 2\,e^- \rightarrow 2\,H_2O_2 \quad [5]$$

Other free radicals produced include the hydroxyl radical ($OH^\bullet$), NO radical ($NO^\bullet$) and peroxyl radical (ROO^-).[33] The generation of ROS increases during hypoxia and reoxygenation and induces serious tissue damage.[31]

In summary neonatal asphyxia is a severe form of neonatal hypoxia with clinical cardiovascular decompensation. Early during hypoxia cardiovascular changes occur in an attempt to maintain perfusion and oxygen delivery to vital organs. However, with ongoing hypoxia and acidosis the myocardium fails and the blood pressure begins to decrease, leading to tissue ischemia and hypoxia. The systemic and regional response to hypoxia depends on the degree and duration of hypoxia and the type of tissue affected. The depletion of oxygen can result in energy shortage and the generation of reactive oxygen species which have detrimental effects on cellular metabolic function.

Table 1-1: Causes of neonatal asphyxia.

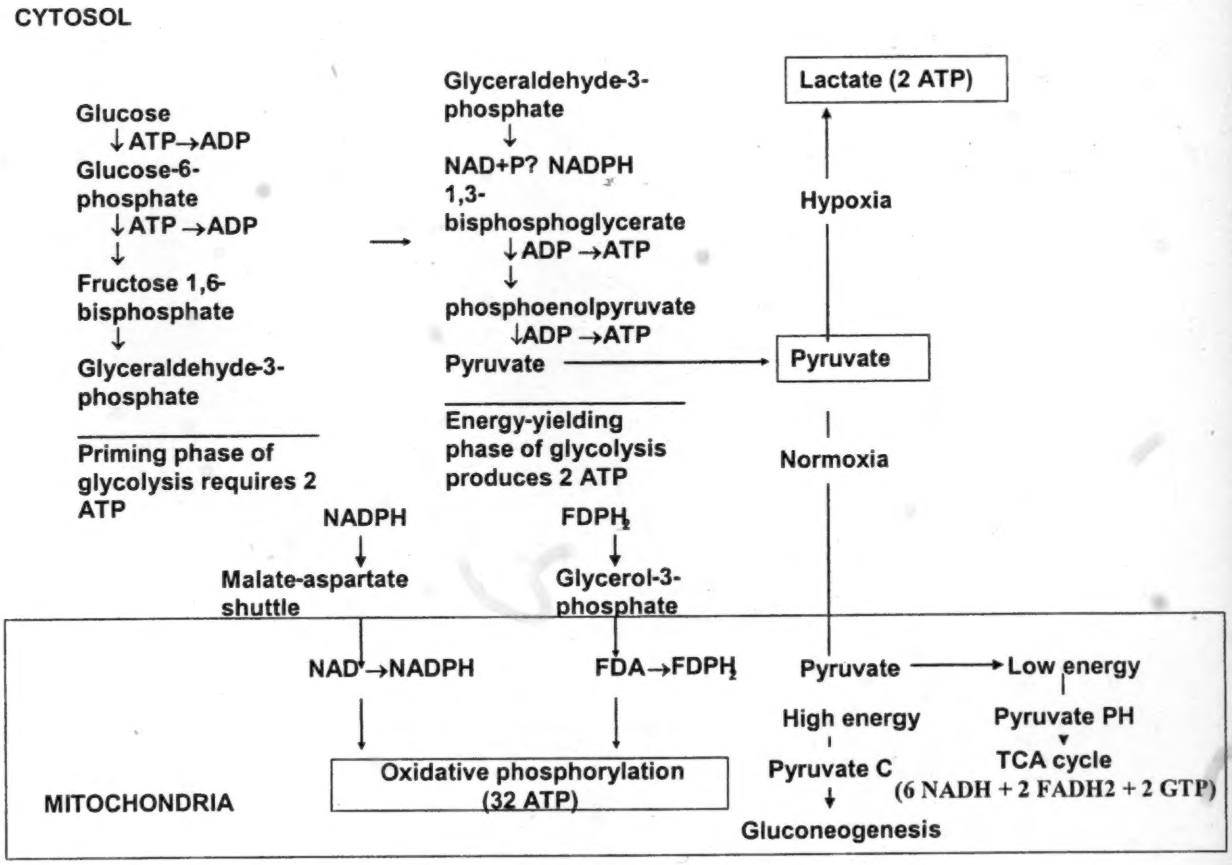
- Perinatal asphyxia
- Severe respiratory distress
- Pulmonary hypertension
- Obstructed airways
- Congenital heart disease and heart failure
- Maternal sedation
- Prolonged labor
- Precipitous delivery

Table 1-2: Complication and system involvement (%) in severe hypoxia.

Cardiovascular (28-78%)	Heart rate and rhythm disturbances Cardiac failure, cardiogenic shock, cardiac stunning Hypotension Myocardial ischemia Patent ductus arteriosus, tricuspid shunting
Pulmonary (26-86%)	Pulmonary hypertension Respiratory distress and failure Pulmonary edema and hemorrhage
Central nervous system (72%)	Increased intracranial pressure, cerebral edema Hypoxic ischemic encephalopathy Seizures, hypotonia, hypertonia Inappropriate antidiuretic hormone secretion
Gastrointestinal tract (29%)	Ileus Necrotizing enterocolitis Intestinal hemorrhage, necrosis and ulceration
Renal (40-70%)	Acute renal failure Acute tubular and cortical necrosis
Metabolic	Hypoglycemia, hypocalcemia, hypomagnesemia Metabolic and lactic acidosis

Fig 1-1: Glucose metabolism and energy production.

Malate-aspartate shuttle and glycerol-3-phosphate shuttle are used to transport NADPH and $FADH_2$ respectively from cytosol to mitochondria.



Pyruvate C: pyruvate carboxylase. Pyruvate PH: pyruvate dehydrogenase. TCA: Tricarboxylic acid cycle. FDA

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Chapter 2

Hypoxia-reoxygenation injury:

Oxidative stress

In 1951 Latham showed that transferring a subject to 100% oxygen following a period of hypoxia paradoxically may worsen the symptoms of hypoxia.[1] This oxygen paradox may be attributed to the generation of oxygen derived free radicals or ROS.[2] Free radicals are compounds containing one unpaired electron in their outer atomic orbits. They are highly unstable and reactive.

Although ROS are normal byproducts of metabolism, increased generation of ROS may result in damage to biological macromolecules. Tissue injury occurs predominantly during the reoxygenation phase when oxygen is introduced to the tissue. Zweier et al showed that ROS synthesis increases seconds after reperfusion.[3] Peroxynitrite (ONOO$^-$) is a cytotoxic byproduct of the reaction between $O_2^{\bullet-}$ and NO, Yasmin et al demonstrated the production of ONOO$^-$, in isolated rat hearts subjected to 20 min of ischemia, peaked at 30 seconds of reperfusion to 175% of baseline.[4]

The cytotoxic effect of ROS has been implicated in a variety of diseases in newborns including hypoxic-ischemic encephalopathy, necrotizing enterocolitis, respiratory distress syndrome and persistent pulmonary hypertension.[5,6]

To fully understand the clinical consequences of neonatal asphyxia, a thorough understanding of the pathophysiology of hypoxia-reoxygenation injury is required.

2-1. Oxidative stress and reactive oxygen production

Oxidative stress was first described by Birch in 1859.[7] He pointed out that the use of oxygen therapy in diseased conditions can produce serious symptoms. He also pointed out that oxygen can exist in not fewer than three allotropic conditions; ozonic, antozonic and neutral. Oxidative stress occurs when the level of oxidants in a system exceeds the capacity of the antioxidants to neutralize them, and thus can lead to oxidative damage in tissue.

Univalent reduction of oxygen is catalyzed by XO to produce $O_2^{\bullet -}$ and divalent reduction of oxygen results in the formation of H_2O_2 (Fig 2-1). The $O_2^{\bullet -}$ radical can trigger the initiation of a series of reaction yielding more potent radical species (Fig 2-2); H_2O_2 is produced when $O_2^{\bullet -}$ undergoes dismutation, a reaction catalyzed by superoxide dismutase (SOD). The oxygen molecule is unable to undergo a direct trivalent reduction but both $O_2^{\bullet -}$ and H_2O_2 can. In the presence of non-protein bound transition metals in the reduced form (e.g. ferrous ion [Fe^{2+}]), H_2O_2 reacts with $O_2^{\bullet -}$ to form the cytotoxic ROS, hydroxyl radical ($OH^{\bullet}$).[8]

Nitric oxide is a free radical produced by endothelial cells, neurons, and activated macrophages and neutrophils. Nitric oxide is an important intracellular messenger and vasoactive agent.[9] At biological concentration it is a non-cytotoxic free radical.[10] However, in the presence of other free radicals, for example NO will react rapidly with $O_2^{\bullet -}$ to generate reactive nitrogen species, such as $ONOO^-$, a highly toxic product of this reaction. The limiting step for this reaction is the concentration of $O_2^{\bullet -}$ which is normally kept low by the enzyme SOD.[10]

2-2. Oxygen is an important component of ROS generation

Several recent studies have demonstrated that ROS are responsible for a major portion of hypoxia-reoxygenation injury in the affected tissue.[11] The huge burst of ROS during reperfusion is well documented.[12] Oxygen is an essential component for ROS and the production of these free radicals may be proportionate to oxygen level during reperfusion. Kaneda et al looked at the effect of arterial oxygen partial pressure during reperfusion on the recovery of cardiac function in ischemic isolated rat hearts.[13] Langendorff perfused rat hearts were subjected to 30 min ischemia and randomized to hypoxic reperfusion, physiologic reperfusion and hyperoxic reperfusion. The recovery rate of cardiac enzymes, cardiac output, systolic pressure, and rate pressure product (a measures of the myocardial oxygen use), were significantly better in the group which received physiologic oxygen level at reperfusion when compared to the other groups. These workers also demonstrated similar findings in patients who underwent either hyperoxic or physiologic cardiopulmonary bypass oxygen tension after aortic unclamping.[13] Patients who received physiological oxygen tension had significantly higher cardiac index and lower blood malondialdehyde (a byproduct of polyunsaturated fatty acid peroxidation) at 6 h postoperatively. Korthuis et al tested the question: is molecular oxygen needed at reperfusion to produce ischemic-reperfusion (I/R) injury?[14] Isolated, maximally papaverine-induced vasodilated canine gracilis muscles were reperfused after 4 h of inflow occlusion with autologous blood equilibrated with either 95% oxygen-5% carbon dioxide or 95% N2-5% CO_2 (PaO_2 3-5 mmHg) gas mixture before normoxia reperfusion. A significantly attenuated I/R injury was seen in the group receiving anoxic blood as indicated by higher total vascular resistance and lower vascular permeability changes compared to the group who received hyperoxic blood. This was consistent with the findings by Perry et al,[15] they showed that mucosal bleeding was higher during reperfusion compared to bleeding during ischemia, and the mucosal bleeding was attenuated by gradual reintroduction of reperfusion or reperfusion with hypoxic blood before introducing normoxic reperfusion.

2-3. Sources of ROS during hypoxia-reoxygenation

Four main sources of ROS generation in hypoxia-reoxygenation injury have been recognized: 1) NO derived free radicals, 2) mitochondrial electron chain free radical generation, 3) the hypoxanthine-xanthine oxidase system, and 4) neutrophils-derived free radicals.

Nitric oxide derived free radicals

Nitric oxide is produced in vascular endothelium and smooth muscle from L-arginine. The reaction is catalyzed by NO synthase.[16] Nitric oxide induces smooth muscle relaxation by activating soluble guanylyl cyclase to produce cGMP from guanine triphosphate. Increased cGMP activates a kinase that subsequently leads to the inhibition of calcium influx into the smooth muscle cell and decreased calcium-calmodulin stimulation of myosin light chain kinase. Increased cGMP also activates phosphatase which can decrease the phosphorylation of myosin light chains. Therefore smooth muscle tension development is decreased and vasodilation occurs.[17] Drugs such as sildenafil that specifically decreases the breakdown of cGMP by inhibiting cGMP-dependent phosphodiesterase potentiate the effects of NO-mediated actions on the target cell.[18]

As mentioned previously NO is a non-reactive, non-toxic free radical at biological concentration, but it will react with other free radicals when present in excess. The most well documented reaction is the generation of the highly toxic $ONOO^-$ (Fig 2-3). Under normal condition the formation of $ONOO^-$ is limited by the small production of $O_2^{\bullet-}$ but during hypoxia-reoxygenation a burst of $O_2^{\bullet-}$ and NO is generated from endothelial cells, macrophages and neutrophils and the removal of $O_2^{\bullet-}$ by SOD is out-competed by the reaction with NO and more $ONOO^-$ is formed.[4,10] Peroxynitrite is not a free radical, but a powerful oxidant. Reported cytotoxic effects of $ONOO^-$ include sulfhydral bond oxidation, tyrosine nitration, lipid peroxidation, and impairment of mitochondrial respiration.[19] Peroxynitrite can be converted to peroxynitrous acid (ONOOH) with subsequent cleavage and release of an intermediate with $OH^{\bullet}$-like activity.[10]

Sulfhydryl bonds in protein and non-protein thiols are critical to the active site of many enzymes and important for maintaining the native conformation of many proteins. They are common targets for free radicals and $ONOO^-$.[20] Tyrosine nitration is assumed to affect the phosphorylation of tyrosine residues in the substrate proteins of tyrosine kinase in cellular signal transduction. Nitrotyrosine, a toxic compound produced through the nitration of tyrosine, is a marker for $ONOO^-$ formation. Nitrotyrosine formation has been implicated in a number of different diseases in the neonate including respiratory distress syndrome, chronic lung disease and necrotizing enterocolitis.[21]

Mitochondrial electron chain free radicals generation

Ambrosio et al first demonstrated that the mitochondrial electron transport chain contributes to the increased generation of ROS during reoxygenation.[22] Mitochondria are a major site of free radical generation as mitochondria contain electron transport chain carriers capable of generating oxidative agents, but these agents are usually well-controlled by the abundant mitochondrial antioxidant defenses.[23] However during hypoxia the depletion of ATP and shortage of NAD^+ can inhibit the normal mitochondrial electron transport allowing for greater electron leakage and increased partial reduction of oxygen molecules which generate $O_2^{\bullet}$ and H_2O_2 (Fig 2-1).[24] Furthermore the electron transport chain may become highly reduced and capable of transferring electrons to any available oxygen, which may occur during reperfusion, to form free radicals.[25]

The mitochondrial electron chain ROS generation is an important mediator of oxidative stress in tissue rich with mitochondria such as cardiomyocytes. Vanden Hoek et al showed that oxidative I/R injury can occur in isolated cardiomyocytes,[26] without the influence of neutrophils or XO and this oxidant generation was increased with the use of mitochondrial electron transport inhibitor and was attenuated with decreasing availability of oxygen.[27]

Hypoxanthine-xanthine oxidase system

Xanthine oxidoreductase is a widely spread cytosolic enzyme, most abundant in the liver and intestine.[28] It exists in two forms; xanthine dehydrogenase (XD) and XO. The former (XD) is the predominant form in the non-ischemic cells and it degrades nucleic acids. It utilizes NAD^+ as an electron acceptor in the oxidation of purines to form xanthine (Fig 2-4). During hypoxia XD is converted reversibly (oxidation of sulfhydryl residue) or irreversibly (proteolytic cleavage) to XO. The enzyme XO uses oxygen as an electron acceptor instead of NAD^+. It generates $O_2^{\bullet-}$ and H_2O_2 by the oxidation of hypoxanthine. Hypoxanthine is formed from hypoxic degradation of adenosine monophosphate.[29] The XO system has been recognized as the most important ROS generating enzyme in hypoxia-reoxygenation injury. Increased plasma concentration of hypoxanthine was found in asphyxiated neonates.[30,31]

Neutrophil-derived free radicals.

There has been a veritable explosion of research demonstrating the role of polymorphonuclear leukocytes in reperfusion injury. The advantage of using leucocyte-depleting filters in an attempt to remove activated leukocytes and to reduce ROS production during reperfusion has been demonstrated in transplant medicine.[32] Most of the initial work done in this area focused on the polymorphonuclear leukocytes mediated reperfusion injury to the myocardium: polymorphonuclear leukocyte activation and subsequent plugging and degranulation, resulting in the release of oxidizing compounds, which are responsible for the no-reflow phenomenon following myocardial ischemia.[33] When polymorphonuclear leukocytes are activated they generate large amounts of H_2O_2 and activate myeloperoxide producing enzymes. Myeloperoxide is found almost exclusively in neutrophils and it is a sensitive marker in neutrophil infiltration and ROS production.[34] A large fraction of the H_2O_2-myeloperoxidase reacts with chloride and other halides to produce their corresponding hypohalous acids,[35] which are capable of damaging a wide range of organic molecules including most of those that make up the structure of the cells and proteinaceous extracellular matrix.[36] The activated neutrophils also release oxidized α-1-proteinase inhibitor that allows the secretion of proteolytic enzymes such as elastase to begin degrading the extracellular matrix, thus destroying capillary integrity and interfering with tissue metabolism and anabolism.[36] The most important activator of neutrophil activity during hypoxia-reoxygenation injury is the XO derived ROS.[34]

2-4. Antioxidant mechanisms

The production of ROS may have a role in normal biological function, especially in granulocytes and other phagocytic cells where the ROS may play a key role in cell-mediated immunity and microbiocidal activity.[35] However uncontrolled production of ROS predisposes the body to a variety of diseases. Indeed oxygen free radical disease in the neonate has been reported by Dr. Saugstad and other.[5,6,11]

Free radicals are controlled within the body by antioxidant mechanisms, which can be found in the intracellular and extracellular compartments. Among these glutathione is an important antioxidant, which forms the largest pool of non-protein thiols in the body and is also a major intracellular antioxidant. Antioxidants are usually classified as non-enzymatic or enzymatic types, which are discussed briefly as below.

Glutathione

Glutathione is a tripeptide thiol-containing compound that found in plasma and tissue and it is formed in the cell from the amino acids: glutamate, cysteine and glycine. It exists in a monomeric reduced form (GSH) and a dimeric oxidized form (GSSG). The glutathione system plays an important role in the detoxification of reactive nitrogen species (Fig 2-4). It has been shown that the administration of high concentration of glutathione to isolated rat hearts subjected to either I/R or peroxynitrite infusion, attenuates the induced mechanical dysfunction.[37,38] Usually $ONOO^-$ is detoxified by glutathione oxidation.[10] Albumin and cysteine are other thiol containing proteins which with the glutathione may convert $ONOO^-$ to nitrosothiols.[10] This detoxification reaction potentially prevents the toxic buildup of $ONOO^-$ and possibly regenerates NO through the nitrosothiols such as nitrosoglutathione or GSSG. Compared to NO, nitrosothiol formation has been suggested as one of the mechanism which cause and prolong the vasodilator effect of NO which has short half life and is rapidly scavenged by the oxyhaemoglobin of red blood cells.[39] Nitrosothiols are more stable in the plasma with a half life of about 40 min, and release NO over a longer period thus prolonging the vasodilator effect of NO.[10]

When mammalian cells are exposed to increased oxidative stress, the Redox ratio (ratio of GSH/GSSG) will decrease as a consequence of GSSG accumulation. The measuring of the GSSG level, or determining the Redox ratio, are useful parameters of oxidative stress and can be used to monitor the effectiveness of antioxidant strategies.

Extracellular chain breaking antioxidants

These are small molecules, which react with free radicals to produce other less potent radical compounds. They include scavengers such as α-tocopherol and ascorbate. α-Tocopherol is present in the cell membrane and plasma lipoproteins, and functions as a chain-breaking antioxidant in the lipid peroxidation by ROS. [40] Once the tocopherol radical is formed, it can migrate to the membrane surface and is reconverted to α-tocopherol by reacting with ascorbate or GSH.[41]

Glutathione peroxidase-reductase

Glutathione peroxidase belongs to the family of selenoproteins. It catalyses the reduction of H_2O_2 (Fig 2-5). Reduced glutathione provides the reducing equivalents for the glutathione peroxidase to reduce H_2O_2 to form GSSG and water.[42] This enzyme is specific for its hydrogen donor, GSH, and nonspecific for the hydroperoxides ranging from H_2O_2, $O_2^{\bullet-}$ to organic hydroperoxides. The GSSG is then recycled by the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) to GSH, a reaction catalyzed by glutathione reductase.

Superoxide dismutase (SOD)

This is a metalloprotein enzyme found in the cell cytoplasm (copper and zinc-containing isoforms), in the mitochondrial matrix (manganese-containing isoforms) and in the extracellular fluid (EC-SOD). Superoxide dismutase catalyzes the destruction of the $O_2^{\bullet-}$ radicals. The biosynthesis of SOD is mainly controlled by its substrate, the $O_2^{\bullet-}$, which is also removed by the glutathione reductase system[43,44]

Catalase

Catalase is a hemoprotein that catalyses the decomposition of H_2O_2 to water and oxygen. It is present in almost all the mammalian cells and it is localized in the peroxisomes or the microperoxisomes.[34]

2-5. Antioxidant mechanisms during the neonatal period

At birth the newborn infant is abruptly exposed to significant higher levels of oxygen at room air compared to that in fetal life. The newborn infant not only needs to withstand the increased demand for energy production but also the increased generation of toxic oxygen radicals, ROS, which can oxidize critical macromolecules. Nozik-Grayck et al showed that there is a developmental regulation of the EC-SOD in the neonatal lungs and the expression of this enzyme decreases during the first week of life.[45] Dr. Saugstad claimed that neonates have reduced defense to the oxidative stress due to the prematurity of the antioxidant systems.[46] The level of certain antioxidants may also differ from those of adults. Lindeman et al showed that plasma concentrations of α-tocopherol, β-carotene and sulfhydryl groups including GSH were lower in cord blood of preterm and term infants compared to adults although the total free radical trapping ability of the newborn was higher than that of the adult.[47] Giles et al showed that prenatal hypoxia disrupted the normal developmental secretion of active EC-SOD in the lung of rabbit.[48] Asphyxia and the use of high oxygen concentration in neonatal resuscitation may therefore present an extra challenge to these infants. Interestingly Vento et al demonstrated an increase in GSSG, SOD and catalase at birth or 72 h after birth in asphyxiated newborns compared to controls.[49] Furthermore level of GSSG, SOD, and catalase at 72 h and 28 postnatal days were higher in infants resuscitated with 100% oxygen compared to those with 21% oxygen.[49]

2-6. Cellular damage induced by oxidative stress

Reoxygenation after hypoxia can induce the production of much larger and potentially damaging amounts of ROS than hypoxia alone by causing an oxidation chain reaction of vital cell components; polyunsaturated fatty acids, proteins, and nucleic acids are common targets. The newly damaged molecules become free radicals which then attack other molecules starting a chain reaction. Oxidative damage can result in (1) loss of enzyme activity and protein cross-linking, (2) alteration of intrinsic membrane properties like fluidity and ion transport, (3) inhibition of protein synthesis, and (4) DNA damage and ultimately cell death. Some of the well known consequences of increased ROS generation include: DNA strand scission,[50] nucleic acid base modification,[51] protein oxidation[52] and lipid peroxidation.[53]

Exposure of DNA to ROS causes extensive strand breakage and degradation of deoxyribose, an effect shown in vitro to be due to formation of $OH^{\bullet}$.[51,52] Neutrophils-derived hypochlorous acid can also induce DNA damage through the chlorination of purine bases in DNA, while $ONOO^-$ can induce the nitration of guanine bases.

The oxidation of Sulfhydryl bonds will inactivate essential enzymes and disturb cellular homeostasis by damaging membrane transport enzyme as mentioned earlier.

Polysaturated fatty acids are essential components of cell, organelles, nuclear and plasma membranes. These structures are targets for lipid peroxidation by ROS. As a result cell lysis can occur due to changes in cell permeability.

The mitochondrial electron transport chain can be inhibited by the ROS and reactive nitrogen species. Structural destruction of the mitochondrial membrane through lipid peroxidation can add to the cellular destructive effect of the free radicals.

2-7. Clinical sequelae of neonatal asphyxia related to ROS.

Hypoxia ischemic encephalopathy

During the initial phase of asphyxia, cerebral blood flow and oxygen delivery is maintained by various mechanisms; cardiac output redistribution, hypercapnia and the increased surge of epinephrine to maintain systemic blood pressure. Autoregulation of cerebral blood flow will maintain the brain perfusion in spite of an initial drop of mean blood pressure. During the early phase of brain injury, brain temperature drops and local release of the neurotransmitter gamma-aminobutyric acid (GABA) increases, transiently minimizing the impact of hypoxia. With prolonged asphyxia, the early compensatory adjustment fails and cerebral blood flow becomes dependent on systemic blood pressure. When cerebral blood flow falls below a critical level, brain hypoxia will occur. Brain hypoxia will lead to intracellular energy failure. Reoxygenation can temporarily correct the cellular energy deficit, but also triggers a chain of events leading to secondary, or delayed energy failure, neural cell swelling and death. These events are manifested clinically as hypoxic ischemic encephalopathy,[54,55] which occurs 6-24 hours after the initial injury. The delayed injury phase is characterized by apoptosis and cell necrosis and may however continue for weeks. The severity of brain injury in this phase correlates well with the severity of long term adverse neurodevelopmental outcome. The delayed phase has been attributed to the action of ROS.[56] The endothelium is thought to be the site of ROS and reactive nitrogen species production.[56]

Nakashima et al[56] showed that free radical formation was involved in the cerebral damage induced by I/R of the middle cerebral artery in rats, and that $OH^{\bullet}$ was responsible for the reperfusion injury after transient focal brain ischemia. Palmer et al found that allopurinol, a XO inhibitor, administered 15 min after the induction of hypoxia ischemia reduced brain injury in 7-day-old rats,[57] while oxpurine, another XO inhibitor, attenuated ROS formation in other studies.[56] Clinically Van Bel et al showed that a high dose of allopurinol had beneficial effects on free radical formation and electrical brain activity, without producing toxic side effects.[58]

Both ischemia and hypoxia increase the release of excitatory amino acids glutamate and aspartate in the cerebral cortex and basal ganglia.[59] Excitatory amino acids cause neuronal death immediately through the activation of receptors for substances such as kainate, N-methyl-D-aspartate (NMDA), and amino-3-hydroxy-5-methyl-4 isoxazole propionate (AMPA). Cell death can be caused by the activation of the ion channel-associated receptors which is related to an increased intracellular concentration of calcium and lipid peroxidation of cell membranes. As a result, this leads to water influx the cell, cell swelling and death. Excitatory amino acids also increase the local release of NO which may exacerbate the neural damage through unclear mechanisms. The regional difference in severity of injury may be explained by the fact that excitatory amino acids particularly affect the CA1 regions of the hippocampus,[60] the developing oligodendroglia, and the subplate neurons along the borders of the periventricular region of the developing brain.[61]

Myocardial stunning

The phenomenon of myocardial stunning refers to prolonged but reversible post-ischemic ventricular dysfunction. Myocardial stunning was first termed by Braunwald and Kloner in 1982.[62] Myocardial stunning related to I/R has been observed in many animal studies. In human the occurrence of myocardial stunning has also been observed in patients with acute myocardial infarction subjected to recanalization therapy. Here the viable myocardium salvaged by reperfusion, may remain stunned with delayed contractile recovery. Myocardial stunning was thought to occur only after I/R but it is clear now that hypoxia-reoxygenation can induce similar injury.[63] Ytrehus et al demonstrated that reperfusion of the isolated rat heart with hypoxanthine and XO produced extensive myocardial necrosis and cellular damage, which was attenuated by the addition of SOD and catalase to the perfusate.[64] The cause of myocardial stunning may be due to the ROS generation that leads to intracellular calcium influx, cellular protein and phospholipid destruction and hence functional disruption of myocytes.

The response of the neonatal myocardium to injury differs from that of adult myocardium, due to different mechanisms of calcium influx and lower antioxidant capacity in the newborn infant heart.[65] Interestingly experimental studies indicate that the newborn infant myocardium develops ischemic contraction earlier than adult myocardium, when subjected to normothermic ischemia without reperfusion, but recovers functionally faster than adult myocardium after short periods of ischemia, or when reperfusion is established before the development of myocardial contraction.[65] These findings indicate unique responses of the immature heart to ischemic or hypoxic injury. This is important in devising better methods of myocardial protection especially during the reoxygenation of the asphyxiated/hypoxic newborn. For example, Ihnken et al demonstrated that reducing oxygen tension during cardiopulmonary bypass attenuated myocardial damage in acute hypoxic piglets.[66] This may be even more important during the early hours of postnatal life when the newborn infant is going through many hemodynamic adaptations.

Necrotizing enterocolitis

Necrotizing enterocolitis (NEC) is a serious gastrointestinal disease in neonates. It is characterized by mucosal or transmucosal necrosis of part of the intestine. The underlying pathology is the accumulation of gas in the submucosal layers of the bowel wall, which progresses to necrosis. Outcomes include eventual necrosis of the bowel wall, perforation, systemic sepsis and sometimes, death. The regions of bowel most often affected are the right side of the colon and the distal ileum, although any portion of the bowel is susceptible. Several factors contribute to the development of neonatal NEC. While prematurity is the most consistent etiological factor, hypoxia and ischemia is a major contributing factor for developing NEC in term and near term infants. Typically term babies will develop NEC in the first week of life.[67] The physiological explanation for the link of hypoxia and NEC is the shunting of blood away from the gut at times of hypoxia (diving reflex) leading to ischemic changes.[68] The histopathological changes of ischemia in NEC may also be secondary to other factors such as inflammatory mediators release, which cause vasoconstriction and lead to ischemia. Okur et al showed that oxidant-mediated lipid peroxidation injury related to free-radical reaction in the gastrointestinal tract of newborn rats plays a central role in mediating hypoxia-induced intestinal necrosis.[69]

Pulmonary hypertension

Euler and Liljestrand were the first to report hypoxic pulmonary vasoconstriction in spontaneously breathing anesthetized cats.[70] This is a mechanism for the control of the distribution of blood within the lungs which automatically increases pulmonary vascular resistance in poorly aerated regions of the lungs, thereby redirecting pulmonary blood flow to regions richer in oxygen content.[71] The mechanism responsible for the initiation of hypoxic pulmonary vasoconstriction is not clear and may be modulated by various mediators such as endothelin,[72] NO and by the autonomic nervous system. Hypoxic pulmonary vasoconstriction may persist despite improved alveolar oxygenation, lung expansion and increased systemic vascular resistance. The role of free radicals, in altering pulmonary vascular tone has been investigated in animal studies. Sanderud et al showed a significant increase in pulmonary pressure and vascular resistance in the piglets after the administration of XO.[73] These changes were exaggerated significantly when both XO and hypoxanthine were administered, while treatment with catalase or allopurinol attenuated the observed response in the pulmonary pressure and vascular resistance. Clyman et al also demonstrated that the addition of hypoxanthine and XO maximally dilate the ductus arteriosus of lambs.[74]

Nitric oxide plays an important role in the pulmonary circulation during normoxic and hypoxic events.[75] In healthy newborn infants, NO concentration changes with postnatal age. Peak nasal NO increased 30% between the ages of 1 to 24 h.[76] Both prostaglandin and NO levels increase after birth and they may be involved in mediating the fall in pulmonary vascular resistance and consequent rise in pulmonary blood flow.

Hypoxia may reduce NO production in the pulmonary circulation. Shaul et al demonstrated that decreased reoxygenation can attenuate basal and activated NO production in fetal pulmonary endothelium but not in the systemic endothelium.[77]

In summary oxidative stress is a normal biological function which may play a key role in cell-mediated immunity and microbiocidal activity. The human body is equipped with antioxidant defense mechanisms which keep ROS production at physiological levels. During the perinatal period there are readjustments of different defense mechanisms including antioxidant activity during the transition from the fetal low oxygen environment to the high oxygen environment of the postnatal life. Over production of ROS during hypoxia and reoxygenation may disrupt the balance between ROS generation and antioxidant activity leading to cytotoxic damage. The sick neonate might also be at an increased risk to oxidative stress due to the decompensation of the antioxidant system in hypoxia-reoxygenation. In addition using high oxygen concentration in the resuscitation of the asphyxiated neonate may be associated with increased ROS generation and higher risk of cytotoxic injury. Asphyxiated neonates often develop sequale like myocardial stunning, pulmonary hypertension, hypoxic ischemic encephalopathy and NEC after the initial resuscitation, which could be related to the ROS injury.

Fig 2-1: Oxygen reduction reactions.

Tetravalent reaction produces complete reduction of oxygen molecule. Divalent and univalent reductions produce partial reduction of oxygen and the generation of free oxygen species, hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\bullet -}$).

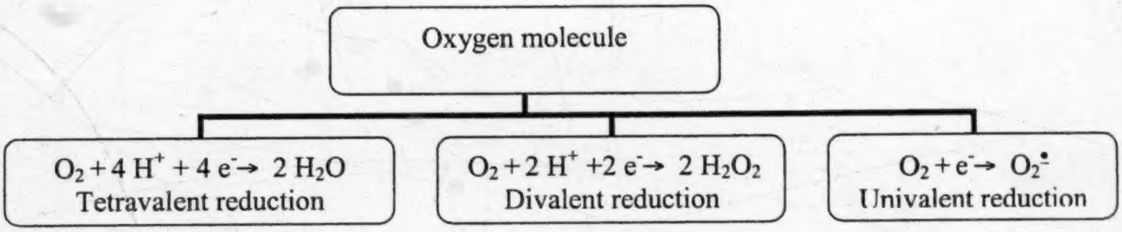
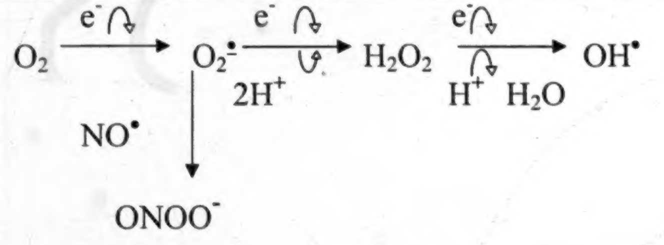

Oxygen molecule
$O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_2O$
Tetravalent reduction
$O_2 + 2\ H^+ + 2\ e^- \rightarrow 2\ H_2O_2$
Divalent reduction
$O_2 + e^- \rightarrow O_2^{\bullet -}$
Univalent reduction

Fig 2-2: The formation of reactive oxygen species.



Superoxide radical ($O_2^{\bullet -}$) formation by univalent reduction of oxygen catalyzed by xanthine oxidase.

$O_2 + e^- \rightarrow O_2^{\bullet -}$

Hydrogen peroxide (H_2O_2) formation by the action of superoxide dismutase [1] and divalent reduction of oxygen [2]

$O_2^{\bullet -} + O_2^{\bullet -} + 2\,H^+ \rightarrow H_2O_2 + O_2$ [1]

$O_2 + 2\,e^- \rightarrow H_2O_2$ [2]

Hydroxyl radical ($OH^{\bullet}$) formation by iron-catalyzed Fenton reaction

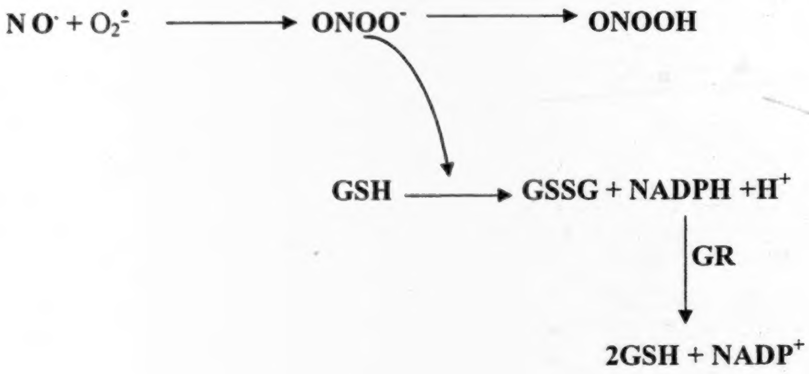
$O_2^{\bullet -} + H_2O_2 \rightarrow OH^{\bullet} + OH^{\bullet} + O_2$ [3]

Peroxynitrite ($ONOO^-$) formation by the reaction between $O_2^{\bullet -}$ and nitric oxide (NO)

$O_2^{\bullet -} + NO \rightarrow ONOO^-$ [4]

Fig 2-3: The formation and detoxification of peroxynitrite.

Peroxynitrite ($ONOO^-$) is formed by the reaction between superoxide radical ($O_2^{\bullet -}$) and nitric oxide (NO). Peroxynitrite is usually detoxified by the oxidation of glutathione (GSH) to form the oxidized form of glutathione (GSSG), which is reduced by NADPH to form 2 GSH. Undetoxified $ONOO^-$ can be converted to peroxynitrous acid (ONOOH).



GP=Glutathione peroxidase. GR= Glutathione Reductase

Fig 2-4: Hypoxanthine-xanthine free radical generation.

During ischemia, the hydrolysis of ATP via AMP leads to an accumulation of hypoxanthine. Increased intracellular calcium enhances the conversion of xanthine dehydrogenase (XD) to xanthine oxidase (XO). Upon reperfusion and reintroduction of oxygen, XO may produce free radicals such as superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2). Xanthine is formed from the hypoxanthine and oxygen reaction. Hydroxyl radicals ($OH^{\bullet}$) could conceivably be produced by the metal catalyzed Haber-Weiss reaction.

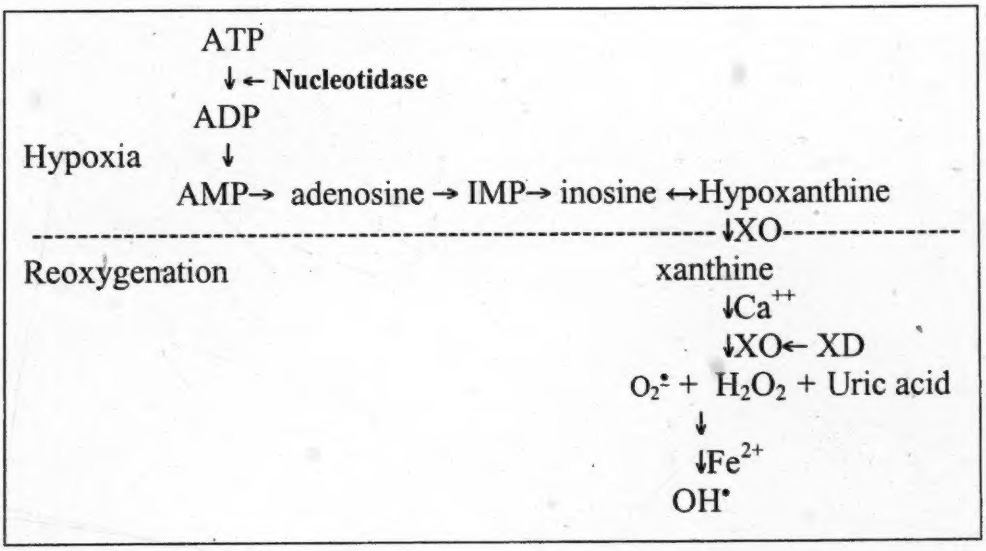
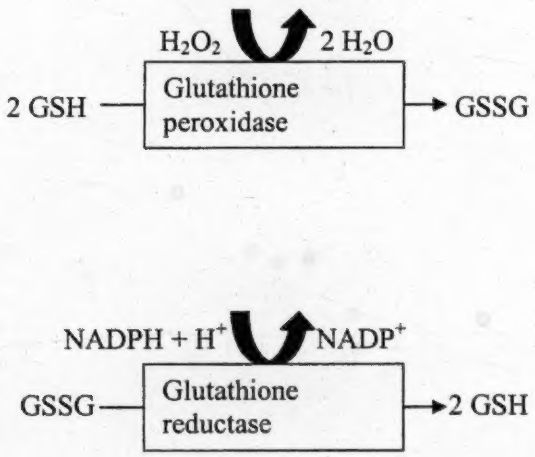


Fig 2-5: Reduction of hydrogen peroxide.

A molecule of hydrogen peroxide (H_2O_2) is reduced to 2 molecules of water (H_2O) while 2 molecules of reduced glutathione (GSH) are oxidized in a reaction catalyzed by glutathione peroxidase. The resulted oxidized glutathione (GSSG) may be reduced to 2GSH in the presence of NADPH, a reaction catalyzed by glutathione reductase.



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Chapter 3

21% versus 100% oxygen in neonatal asphyxia:

21% vs. 100% reoxygenation

Although resuscitation procedures of the newborn infant have been recently revised, the optimal concentration of supplemental oxygen in neonatal resuscitation is still controversial as current clinical data is insufficient to justify using 21% oxygen for resuscitation.[1] But when oxygen is not available, resuscitation with 21% oxygen is reasonable when positive pressure ventilation is required. Effective ventilation with air usually results in hemoglobin that is almost fully saturated with oxygen. However, in severely acidotic states, the beneficial effect of the right-ward shift of the oxygen-hemoglobin dissociation curve decreases[2] and oxyhaemoglobin does not dissociate at the tissue level until the oxygen partial pressure is much lower than normal. Under these circumstances, oxygen dissolved in serum may become an important oxygen source for tissues. Intuitively, it seems like a good idea to give as much oxygen as possible when there is poor ventilation or perfusion. However, oxygen derived free radicals or ROS may be abundantly produced in the hypoxia-reoxygenation process, thus accounting for at least part of the resulting damage.

Several clinical studies examined the short and long-term effects of high oxygen in premature[3,4,5] and asphyxiated infants[6,7,8,9,10,11] (Table 3-1). The result indicate that 21% oxygen is mostly as efficient as 100% oxygen for resuscitation of newborn infants with no differences in growth and neurodevelopment between infants resuscitated with either 21% oxygen or 100% oxygen at 18-24 months postnatal life.[11] In addition 100% oxygen resuscitation at birth can significantly delay the time to first breath, prolong the duration of resuscitation by a median of 2 min and can also augment markers of oxidative stress for weeks after birth compared to resuscitation with 21% oxygen.[7]

A recent Cochrane review acknowledges that there is growing evidence from both animal and human studies that 21% oxygen is as effective as 100% oxygen and that a reduction in mortality has been seen in infants resuscitated with 21% oxygen with no evidence of harm (NNT 20, CI 12-100). However, the small number of identified studies and the use of back-up 100% oxygen in more than a quarter of infants randomized to 21% oxygen made the interpretation of the results inconclusive. The authors indicated that on the basis of currently available evidence, if one chooses 21% oxygen as the initial gas for resuscitation, supplemental oxygen should continue to be made available.[12]

There is insufficient evidence at present on which to recommend a policy of using 21% oxygen over 100% oxygen, or vice versa, for newborn resuscitation. But the search for an answer continues and two recent systematic reviews and meta-analyses have been published. The first one by Davis et al concluded that for term and near-term infants, air should be used initially, with oxygen as backup if initial resuscitation fails, and that the effect of intermediate concentrations of oxygen for resuscitation needs to be investigated.[13] While Saugstad et al showed that neonatal mortality was significantly reduced when depressed newborn infants were resuscitated with ambient air instead of pure oxygen,[14] for infants with low 1-min Apgar score of < 4, no significant difference in neonatal mortality was found although apparent recovery was faster in infants resuscitated with 21% than 100% oxygen.

Neonatal animal studies are summarized in Table 3-2. It has been shown that using 100% oxygen to resuscitate newborn piglets with pneumothorax had no advantage compared to 21% oxygen and was associated with early neurological impairment.[15] More ROS is generated in the myocardium, lung,[16] brain[17] and leukocytes[18] that may increase tissue injury,[19] when 100% oxygen is used compared to 21% oxygen. But when the markers of tissue injuries were studied, resuscitation with 21% or 100% oxygen has been reported to produce similar changes in peripheral flow,[20] myocardial troponin I[21] and oxidative stress markers.[22] In addition the long term effect of 21% oxygen resuscitation on the developing systems of the sick neonate and whether reoxygenation with 21% oxygen is equally efficient in normalizing pulmonary vasoconstriction after hypoxemia are still unclear.[23]

In summary, clinical and animal studies have shown that 100% oxygen is associated with increase oxidative stress and ROS generation which have been implicated in many neonatal disorders. Studies also showed that 21% is as efficient as 100% oxygen for the resuscitation newborn infant. However the long and short term effects of 21% and 100% reoxygenation on the developing systems of the newborn during hypoxia-reoxygenation, especially the systemic and regional circulation is not known. Inotropes are commonly used to support cardiovascular decompensation in these infants, but the effect of 21% and 100% reoxygenation on the systemic and regional responses to these drugs is not clear. In the following chapter, the control mechanisms of the cardiovascular system will be discussed.

Table 3-1: Summary of the clinical studies of 21% oxygen versus 100% oxygen in neonatal resuscitation.

Authors (Year)	Number allocated	Major finding
Ramji S[6] (1993)	84 (Single center)	No differences between the groups, except the group reoxygenated with 21% had higher median Aapgar scores at 5 min, lower duration of assistant ventilation and shorter time to first cry.
Saugstad O[7] (1998)	609 (Multicenter)	No differences in mortality and/or moderate or severe hypoxic ischemic encephalopathy, except the group reoxygenated with 21% had higher Apgar score at 1 min and shorter time to first breath and first cry.
Vento M[8] (2001)	830 (Single center)	No significant differences in the effectiveness of either gas sources on the final outcome, except the 21% reoxygenation group had a shorter time to attain spontaneous respiration. The group resuscitated with 100% oxygen showed significant oxidative stress at 28 days of postnatal life.

Authors (Year)	Number allocated	Major finding
Vento M[9] (2003)	106 (Single center)	No differences in outcome except the 21% oxygen group needed less time of ventilation for resuscitation. At 15 min GSH was decreased and GSSG, the glutathione cycle enzymes, and SOD activities were increased in both asphyxiated groups, however the 100% oxygen resuscitated group showed significantly greater alteration that correlated positively with hyperoxemia.
Ramji S[10] (2003)	431 (Multicenter)	No differences in mortality, hypoxic ischemic encephalopathy and other acute outcome measures, except for a shorter duration of resuscitation in the room air group.
Saugstad O[11] (2003)	213 (Multicenter)	No differences in somatic growth and neurodevelopmental outcomes at follow up ages between 18 and 24 months.

Table 3-2: Summary of the animal studies of 21% oxygen versus 100% oxygen in neonatal resuscitation.

Authors (Year)	Species	Major Finding
Poulsen JP[22] (1990)	Piglets 1-2 wks old. Alveolar asphyxia.	Circulating concentrations of oxypurines increased in the plasma , CSF and vitreous fluid in both groups during hypoxia and reoxygenation.
Rootwelt T[20] (1996)	Piglets 2-5 D old. Alveolar asphyxia.	Higher oxygen delivery to the intestine and pancreas at 5 min of reoxygenation in 100% group, with no other differences in regional flow.
Kondo M[16] (2000)	Piglets 24 h old. Alveolar asphyxia.	Reduced lung surface chemiluminescence of reactive oxygen species in 21% reoxygenation compared to 100% group.
Temesvari P[15] (2001)	Piglets 3-6 h old.(1.12-1.42 kg). Experimental pneumothorax-induced asphyxia.	Blood Levels of oxidative stress indicators and cerebral histopathology were not different between groups. Early neurological outcome in the 21% group was better than 100% groups.

Authors (Year)	Species	Major Finding
Kutzsche S.[18] (2001)	Piglets 2-4 D old. (1.13-2.1 kg).	100% reoxygenation had higher increase in mean arterial blood pressure, cerebral venous O_2 saturation and arterial blood and cerebral venous H_2O_2 concentration in leukocytes.
Munkeby BH[19] (2004)	12-36 h old piglets. Alveolar asphyxia.	Higher cerebral extracellular glycerol and MMP-2 mRNA levels but lower radical absorbance capacity in the 100% reoxygenation group
Fugelseth D[21] (2005)	Piglets 12-36 h old. (1.7- 2.7kg). Alveolar hypoxia.	No differences in cardiac troponin I level, and cardiac index and pulmonary artery response between groups.
Haase E[23] (2005)	Piglets 1-3 D old. (1.5-2.1 kg). Alveolar hypoxia.	Transiently higher cardiac index and lower systemic vascular resistance in the 21% group. Pulmonary artery pressure normalized in an oxygen-dependent fashion.

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Chapter 4

Cardiovascular function during neonatal hypoxia and reoxygenation:

Adrenergic and dopamine receptors

4-1. Control mechanisms in cardiovascular function

Cardiac performance and vascular tone at any time are the result of the integration of neural, hormonal and local control mechanisms.[1]

The neural control of cardiac output and systemic vascular resistance plays a major role in the moment-by-moment regulation of arterial pressure, whereas hormones are predominant in the long-term regulation of arterial pressure. Several hormones act as effectors for the regulation of salt and water balance and blood volume. These include arginine vasopressin (antidiuretic hormone), angiotensin II, aldosterone, and atrial natriuretic peptide. Central blood volume and arterial pressure are normally maintained within narrow limits by the neural and hormonal mechanisms. The local control mechanisms include the release of vasodilators (e.g. NO) and vasoconstrictors (e.g. endothelin) to control the microvasculature.

The neural control involves sympathetic (thoracolumbar outflow) and parasympathetic (craniosacral outflow) branches of the autonomic nervous system. Each branch consists of a control center, efferent (motor or output from control centre to the effector) and afferent (sensory or input from effector to control center) pathways and is regulated by reflex input and feedback from the medulla oblongata and the supramedullary neuron network.[1]

Vascular tone is determined by the balance of competing vasoconstrictor and vasodilator influences. These influences can be separated into extrinsic factors which regulate arterial blood pressure by altering systemic vascular resistance, and intrinsic factors which are important for local blood flow regulation within an organ. Extrinsic factors (neurohumoral) such as sympathetic nerves and circulating angiotensin II increase vascular tone (however, some circulating factors such as atrial natriuretic peptide decrease vascular tone). Intrinsic factors are mainly myogenic which can either increase or decrease tone and include; endothelial factors (NO, endothelin), local hormones/chemical substances (arachidonic acid metabolites, histamine and bradykinin) metabolic by-products and hypoxia. The mechanisms by which the above factors influence the vascular tone involve a variety of signal transduction mechanisms that ultimately influence the interaction between actin and myosin in the smooth muscle. The vascular effect of various neurotransmitters and hormones may also be mediated by intrinsic factors, for example the vasodilatory response to adenosine and acetylcholine is partly mediated through endothelium-derived relaxing factors (EDRF), hyperpolarizing factors (EDHF) and contracting factors (EDCF).[2] Adenosine is an endogenous metabolite of ATP which is produced and released in various tissues in response to a number of physiological and pathological conditions, including hypoxia. Local factors are also involved in the control of regional blood flow. Studies on the intestinal vasculature of the newborn piglet showed that myogenic factors predominate in animals more than 1 day old.[2]

In summary the cardiac performance and vascular tone at any time is the result of the integration of neural, hormonal and local mechanisms. Chemoreceptors and mechanoreceptors relay the metabolic changes induced by hypoxia to the control centers in the brain stem. As a result, redistribution of blood flow and regional vascular responses occur. The resultant response is controlled by feedback mechanisms and higher centers. There are regional variations in the response to hypoxia to maintain adequate flow to the vital organs for as long as possible. When arterial pressure drops, the generalized vasoconstriction caused by the baroreflex spares the brain and heart, allowing flow to these two vital organs to be maintained. Under controlled respiratory conditions in laboratory experiments, stimulation of the chemoreceptors results in higher parasympathetic drive (bradycardia, coronary vasodilation), increase in secretion of adrenal catecholamines and decrease in sympathetic drive (peripheral vascular vasoconstriction) and vasodilation in the circulatory bed of the brain and the heart circulation.

4-2. Adrenergic and dopamine receptors

Oxygen and catecholamines are used commonly for the support of the cardiovascular system in sick newborns. However the hemodynamic effects of the catecholamines during hypoxia and hyperoxia are not well defined in this population. Synthetic catecholamines, dopamine and epinephrine, are commonly used in neonatal intensive care. These drugs have different modes of action which may result in varying systemic pharmacological effects including positive chronotropy and inotropy (via β-adrenergic receptors), and reduced or increased vascular resistance (via β- and α-adrenergic receptors respectively). The mechanism of action is often dose and receptor dependent. The maturation of these receptors may further affect the response. In this chapter the structures and functions of the adrenergic and dopamine receptors will be described.

Adrenergic receptors

Neurotransmitters released from nerve terminals of the autonomic nervous system bind to specific receptors which are specialized macromolecules embedded in the cell membrane called adrenergic receptors (AR) (Table 4-1) or adrenoreceptors.[3] These receptors are classically postsynaptic receptors which are located either near the effector organ or on the cells of effector organs even in the absence of sympathetic innervations. Adrenergic receptors can be divided into two major types; alpha (α-AR) and beta (β-AR) receptors.

Alpha adrenergic receptors

The α-AR are subdivided into the α_1-AR (selectively blocked by prazosin), or the α_2-AR (blocked by yohimbine). Molecular biology has further subdivided the α_1 receptors into α_{1A}, α_{1B}, α_{1c} and the α_2 subdivided into α_{2A}, α_{2B}, α_{2C}.The most predominant α_1-AR subtype in the heart and vasculature are the α_{1A}-AR. Activation of the α_{1A}-AR mediates increased inotropy, hypertrophy of cardiac myocytes, and constriction of arteries and veins.[4] The α_2-AR mainly mediates increased blood pressure through peripheral vasoconstriction.

Beta adrenergic receptors

The β-AR are divided into β_1-AR (selectively blocked by metoprolol), β_2-AR (blocked by labetalol) and β_3-AR. Beta AR when activated, generally produce dilatation of smooth muscle and excitation of cardiac muscles. β_1-AR receptors are predominant in cardiac tissue and produce chronotropic and inotropic responses, and lipolysis when activated, whereas activation of β_2-AR produces bronchodilation, vasodilation and uterine relaxation. β_3-adrenergic receptors activation induces lipolysis in adipose tissue. Beta-AR are found in different combinations in various organs throughout the body (table 4-2) which may explain in part the differential response of the regional circulation to stimulation by catecholamines.[5]

Adrenergic receptor activation and signal transduction mechanism

Adrenergic receptors are transmembrane spanning G-protein coupled receptors. Each of the receptor sub-types spans the membrane seven times with each spanning region comprising 20-28 hydrophobic amino acids. These membrane-spanning helices are connected by alternating extracellular and intracellular loops. The extracellular part of these receptors is primarily concerned with ligand binding, and the intracellular loops mediate the interaction of the receptor with various signaling and regulatory proteins, such as G-proteins, and G-protein coupled receptor kinases. The activation of G-protein can mediate an inhibitory or a stimulatory effect.

The adenyl cyclase system is involved in the activation of α_2- and β-AR, and cAMP is the second messenger (Fig 4-1) that leads to enzyme phosphorylation mediating the positive inotropy and peripheral vasodilation resepectively.[6] On the other hand, the activated α_1-AR stimulates phospholipase C which generates inositol triphosphate and diacylglycerol as second messengers (Fig 4-2) to cause an increase in cytoplasmic calcium, resulting in vascular smooth muscle contraction and the cardiac inotropic effect.[6]

The effect of epinephrine is terminated by re-uptake into the presynaptic neuron and metabolic inactivation by monoamine oxidase B and catechol-o-methyltransferase.[11]

Changes in adrenergic receptors during the postnatal period

Animal studies have demonstrated that the response to α_1-AR stimulation changes with age. Postnatally, the chronotropic response of cardiac α_1-adrenergic activation changes from stimulatory to inhibitory in most mammals. In neonatal rats, α_1-AR stimulation causes an increased L-type calcium current mediated by protein kinase C phosphorylation of calcium channel proteins, while the stimulation of α_1-AR in young and adult rats results in the reduction of the transient outward K+ current, but does not affect calcium currents.[7]

Although the proportion of β-AR subtypes is the same in both neonatal and adult rats, the β_2-AR may play a more important role in mediating the response to catecholamines in the neonatal rat.[7] In the neonatal rat myocytes, β_2-AR stimulation leads to increased intracellular cAMP accumulation and enhanced phosphorylation of phospholamban and troponin I, responses similar to those produced by β_1-AR stimulation in the adult heart. This developmental change is mainly due to changes in the G_s/G_i protein concentration ratio. The G_s/G_i proteins ratio markedly increases during postnatal development in the rat, mainly due to a relative reduction in the concentration of G_i protein. Whether these findings in the small rat heart can be applied to the human neonate remains unknown.[7]

Effect of hypoxia on adrenergic receptors

Hypoxia can activate the sympathetic nervous system and is associated with increased release of circulating norepinephrine and AR desensitization.[8] Hypoxia-induced AR desensitization is associated with a reduction in β_1-AR density, secondary to the reduced concentrations of β-AR mRNA and increased concentration of G_i proteins in the myocyte sarcolemma.[7] Experimental studies have also shown that chronic hypoxia can produce a decrease in the density of α_{1A}-AR and myocyte concentration of α_{1C}-AR mRNA.[7, 9]

Dopamine receptors

Dopamine is a catecholamine neurotransmitter found in neurons of both the central and peripheral nervous systems. It is stored in vesicles in axon terminals and released when the neuron is depolarized. Dopamine plays an important role both centrally and peripherally. Changes in central dopamine neurotransmission are implicated in processes as diverse as muscle rigidity, hormonal regulation, thought disorder and cocaine addiction. Peripheral dopamine receptors mediate changes in blood flow, glomerular filtration rate, sodium excretion and catecholamine release.[9]

Dopamine receptors (DR) are of two main subtypes DR1 and DR2 (table 4-3). DR1 binds to $G_{\alpha s}$ and DR2 binds to $G_{\alpha i}$ protein.[10] Molecular biology techniques resulted in the identification of five pharmacologically distinct dopamine receptor subtypes, D1, D2, D3, D4, and D5. The D1 and D5 receptors are classified as members of the DR1 subfamily and sometimes refer to as D1-like receptors. The D2, D3 and D4 receptors are classified as members of the DR2 subfamily or D2-like receptors.

Dopamine receptor activation and signal transduction mechanism

Dopamine interacts with specific transmembrane spanning G-protein coupled receptors (seven-transmembrane-spanning subunits). The effect of dopamine is terminated by re-uptake into the presynaptic neuron via a dopamine transporter or by metabolic inactivation by monoamine oxidase B and catechol-o-methyltransferase.[11] The interaction of dopamine with its receptors initiates a conformational change in the receptor, which allows it to combine with its associated G-protein ($G_{\alpha s}$ or $G_{\alpha i}$).[12] This will lead to activation of adenyl cyclase and the production of cAMP which in turn acts on protein kinase resulting in a variety of responses in the cell.[12]

Changes in dopamine receptors during the postnatal period

Animal studies showed postnatal changes in the expression of these receptors in different tissue. Tomassoni et al investigated the postnatal development in the expression of D1-like and D2-like receptors in peripheral blood lymphocytes, frontal cortex striatum and hippocampus of male Wista rats; Dopamine D1-like and D2-like receptor maturation in frontal cortex, hippocampus and striatum revealed an increased receptor density until the 4th week of age and a relative stabilization of receptor density values between the 4th to the 12th week. The density of the lymphocyte D1-like receptors increased from the 1st to the 3rd week of age, remained constant from the 3rd to the 8th week of age, and then increased slightly at 12 and 16 weeks of age. The lymphocyte D2-like receptors postnatal maturation was similar to that of the central type.[13]

Effect of hypoxia on dopamine receptors

Labaune et al looked at the effect of hypoxia on the expression of DR in rabbit's adrenal glands.[14] They showed that hypoxia decreases DR1 mRNA expression level in newborn rabbits, whatever the duration and severity of hypoxia. This down-modulation was not observed in 25-day-old and in adult rabbits. Dopamine has a well-established role as having an inhibitory modulator effect on the carotid body. Pedersen et al showed that DR2 blockade increases the response of the carotid body to hypoxia and that dopamine infusion inhibited the carotid body responses to hypoxia.[15]

In summary the adrenergic and dopamine receptors play an important role in the neural control of cardiovascular functions. The activation of these receptors can result in varying systemic and regional effects. The effect of age and hypoxia on the expression of these receptors is well documented in animal studies, but whether these changes can be applied to the human neonate remains unknown.

4-3. Pharmacology of epinephrine and dopamine

Epinephrine

Natural epinephrine is a catecholamine hormone. It is a sympathomimetic monoamine derived from the amino acids phenylalanine and tyrosine. Endogenous epinephrine is formed from norepinephrine (Fig 4-2). The chemical formula of epinephrine is $C_9H_{13}NO_3$.[16] Epinephrine was first isolated and identified in 1897 by John Jacob Abel. Jokichi Takamine discovered the same hormone in 1901, without knowing about the previous discovery, and called it adrenaline. Friedrich Stolz first artificially synthesized it in 1904 (Fig 4-3).[17]

Physiologic effects

Epinephrine is principally produced with stress and causes widespread metabolic and hemodynamic effects via effects on α and β-AR. The effects of epinephrine depend on the dosage selected and the range of plasma concentration achieved in the individual patient (Table 4-4). Generally epinephrine infusion at low doses (0.02-0.2 mcg/kg/min) primarily induces β-AR stimulation, with increased cardiac output and mild vasodilation. β_1-AR are most sensitive to epinephrine, and are affected by very low plasma concentrations resulting in inotropic and chronotropic effects that may also increase myocardial oxygen consumption. Stimulation of β_2-AR leads to vasodilation of splanchnic and skeletal muscle beds. At 0.2-2 mcg/kg/min, the effect is mixed α- and β-AR stimulation, causing increased cardiac output and increased systemic vascular resistance. Epinephrine doses more than 2 mcg results in mainly α-AR stimulation which tends to mask the β_1-AR cardiac effects due to the intense vasoconstriction effect. The β-AR effects outlast the α effects so secondary hypotension may occur after a bolus or upon termination of an infusion of epinephrine. However vasoconstriction from α-AR stimulation in cutaneous and renal vascular beds occurs at all concentrations, whereas at higher concentrations, vasoconstriction effects in the pulmonary, splanchnic, skeletal muscle, cerebral, and coronary vascular beds predominate. At high concentration of epinephrine, myocardial irritability may occur, manifested by atrial and ventricular dysrhythmias.

Metabolic effects occur at high plasma concentrations of epinephrine. These include hyperglycemia from α AR mediated suppression of insulin release that leads to ketogenesis, gluconeogenesis, and accelerated glycogenolysis. Lactic acidemia will occur. Hypokalaemia is attributable to β_2 AR linked to Na+-K+ ATPase in skeletal muscle. Other effects include hypophosphatemia, and activation of lipase.[18]

Adverse effects

Adverse effects of epinephrine administration include increased myocardial and global oxygen consumption, tachycardia and hypertension. The extent to which the increased oxygen utilization is balanced by improved coronary blood flow depends on the state of the myocardium. Epinephrine increases pulmonary vascular resistance. In addition, pulmonary arterial and venous pressures increase because of increased systemic to pulmonary shunt, which can lead to pulmonary edema. High doses induce widespread vasoconstriction that may terminate in hypertensive crisis, renal failure, and gangrene of distal extremities. Infiltration into local tissues or intra-arterial injection can produce severe vasospasm and tissue injury. If extravasation is followed by impaired local perfusion, local injection of the α-AR antagonist phentolamine can be used.[18]

Metabolism

Epinephrine is rapidly distributed to the heart, spleen, several glandular tissues and adrenergic nerves. It is approximately 50% bound to plasma proteins. The onset of action is rapid and after intravenous infusion the half-life is approximately 5-10 min. Epinephrine is rapidly metabolized in the liver and tissues. Up to 90% of the intravenous dose is excreted as metabolites in the urine (Fig 4-2) Vanillylmandelic acid and 3-methoxy-4-hydroxyphenylglycol.[16,18]

Dopamine

The chemical formula of dopamine is $C_8H_{11}NO_{22}$ (Fig 4-4). The synthetic dopamine, dopamine hydrochloride, can stimulate α and β AR and DR. Dopamine do not cross the blood-brain barrier and so does not activate dopamine receptors in the brain.

Physiologic effects

Dopamine interacts with specific membrane receptors to produce its effects by direct action on α and β- AR as well as DR[10]. These effects are terminated by re-uptake into the presynaptic neuron by a dopamine transporter or by metabolic inactivation by monoamine oxidase B or catechol-o-methyltransferase. The cardiovascular effect of dopamine is dose-dependent according to conventional theories (Table 4-5). At infusion rates of 0.5-2 mcg/kg/min, DR receptors are selectively activated and blood pressure either does not change or decreases slightly. The most reported effects at this rate are renal and mesenteric vasodilatation.[19] Renal plasma flow, glomerular filtration rate and sodium excretion usually increase. At infusion rates of 2-10 mcg/kg/min β_1-AR are activated and cardiac output and systolic blood pressure increase. The total peripheral resistance is relatively unchanged because peripheral vasoconstriction (α-AR effect) balances out vasodilatation (β-AR effect). At infusion rates above 10 mcg/kg/min, α-AR are predominantly activated, causing vasoconstriction, and both systolic and diastolic pressures increase.

Adverse effects

Dopamine may decrease the ventilatory response to hypoxia and hypercarbia by depressing peripheral chemoreceptors response to hypoxia.[20,21] It has similar effects to epinephrine on insulin secretion and glucose metabolism. Since dopamine promotes the release of norepinephrine from synaptic terminals, it can cause severe limb ischemia even at low dose infusion; this risk is particularly increased with extravasation or presence of an arterial catheter.[22]

Metabolism

Dopamine is a rapidly acting compound. Cardiovascular effects are usually evident within 10 min. The inotropic response is diminished in neonates compared with older children and adults due to maturational differences in norepinephrine stores. The duration of action of dopamine is less than 10 min. The half-life of dopamine is approximately 1.75 min after an intravenous bolus due to rapid metabolism and excretion. Dopamine is widely distributed in the body. It is rapidly metabolized and excreted. It is metabolized in the liver, kidneys and plasma. Metabolites are excreted by the kidneys. The major routes of metabolism are deamination by monoamine oxidase and formation of methylated and reduced derivatives by catechol-o-methyl transferase. Following intravenous administration, 75% of the infused dopamine is rapidly converted into metabolites of dopamine and 25% is synthesized into noradrenaline and its metabolic products. The principal metabolites of dopamine are 3, 4-dihydroxyphenylacetic acid and homovanillic acid (Fig 4-2). The clearance of dopamine varies widely in the pediatric population, depending on the developmental age. Its elimination half-life is approximately 2 min in full-term neonates and older children, and may be as long as 4-5 min in preterm infants.[23]

4-4. Use of epinephrine and dopamine in the treatment of shock in neonates. (Review of literature)

Clinical studies

Pellicer et al studied the effect of dopamine (2.5-10 mcg/kg/min) and low dose epinephrine (0.125-0.5 mcg/kg/min) on brain hemodynamics in preterm infants treated for the support of systemic hypotension.[24] They showed that low dose epinephrine is as effective as low-moderate dose of dopamine in increasing mean arterial pressure. At these doses, both inotropes improved cerebral perfusion, as indicated by the increase in both cerebral blood volume and oxyhaemoglobin.

Osborn and colleagues compared the effects of dobutamine with dopamine on systemic blood flow in preterm infants with hypotension; using superior vena cava flow as a surrogate to quantitate systemic blood flow.[25] The authors concluded that although dobutamine produced a greater increase in blood flow than dopamine, there was insufficient evidence of improved clinical outcomes to recommend a single best strategy for cardiovascular support of preterm infants.

In a retrospective study Heckman et al concluded that the infusion of epinephrine (0.05 and 2.6 mcg/ kg/min) increased the systemic blood pressure and the heart rate without decreasing urine output in premature infants ≤ 30 gestation weeks with hypotension not responding to a dopamine infusion up to 15 mcg/kg/min. A potential adverse effect with epinephrine was an increase in metabolic acidosis.[26]

Zhang et al noted that in a portion of preterm neonates, dopamine increases blood pressure by predominantly increasing vascular resistance (vasopressor effect) which was associated with reduced perfusion to the bowel.[27] To demonstrate the presence of selective vascular responses to dopamine in the preterm human neonate, Seri et al reported that low dose dopamine (6.1 ± 3.0 mcg/kg/min) used to treat oliguria or low perfusion in non-hypotensive infants, significantly increased blood pressure and urine output,[28] while mesenteric and cerebral blood flow remained unchanged during dopamine treatment. The increase in renal blood flow was independent of the blood pressure changes. The authors speculated that there is a selective vascular response to dopamine with the functionally mature renal but not mesenteric vasodilatory dopaminergic response in the preterm neonate. The observations also indicate the lack of an effect of low- to medium-dose dopamine on cerebral hemodynamics in the non-hypotensive preterm neonate.

Phillipos et al compared the effect of epinephrine and dopamine in infants >1750 g and found that both epinephrine (0.125- 0.5 mcg/kg/min) and dopamine (5-20mcg/kg/min) increased heart rate and mean blood pressure.[29] Dopamine caused a 10% drop in left ventricle output secondary to a drop in stroke volume while epinephrine increased left ventricular output by 10% due to increased in stroke volume. Both drugs however increased mean pulmonary pressure significantly compared to baselines.

In a randomized, double blind, crossover trial, Roze et al demonstrated that dopamine (12±1.8 mcg/kg/min) was more effective than dobutamine (17±1.7 mcg/kg / min) at increasing mean blood pressure among preterm neonates (≥32 gestation weeks).[30] By following the changes in stroke volume, cardiac output, and calculated vascular resistance, they found that dopamine increased blood pressure by increasing systemic vascular resistance while maintaining stroke volume. On the other hand, dobutamine increased mean blood pressure primarily by inducing significant increases in stroke volume with no effect on systemic vascular resistance.

It was noted by Bhatt-Mehta et al that there was no relationship between dopamine pharmacokinetics and gestational age, postnatal age or birth weight as well as substantial inter- individual variation in dopamine pharmacokinetics in seriously ill infants.[31] In a randomized double blind protocol, DiSessa et al reported increased cardiac performance and systolic blood pressure (but not diastolic blood pressure) with the use of low dose dopamine in severely asphyxiated infants compared to placebo.[32] Paradisis et al studies the use of epinephrine vs. placebo and other treatment in preterm infants with cardiovascular compromise. The authors concluded that there are insufficient data on the use of epinephrine infusions in preterm infants with cardiovascular compromise, and on the effect of epinephrine on morbidity and mortality in preterm infants with cardiovascular compromise to recommend a practice.[33]

The question of what inotropes should be used in different clinical scenarios is still unanswered. A recent Cochrane systematic review on the use of dopamine in term infants with suspected perinatal asphyxia concluded that there is currently insufficient evidence from randomized controlled trials that the use of dopamine in term infants with suspected perinatal asphyxia improves mortality or long-term neuro-developmental outcome.[34] The effects of dopamine versus dobutamine have also been examined in a systematic review.[35] Dopamine was more effective than dobutamine for treatment of systemic hypotension in the short term. However, there is insufficient evidence concerning long-term benefits and safety to give firm recommendations on the choice of drug to treat neonatal hypotension.

Animal Studies

Although there are differences in the manner in which a newborn's body responds to hypoxic insults comparing to older children and adults, most of the pharmacological knowledge of drugs such as oxygen, dopamine and epinephrine used to support these infants, was extrapolated to the newborn from adult studies. The immature organ systems in the neonate may not be capable of metabolizing or neutralizing the drugs as effectively as the mature systems. Exposure of the developing organs to chemicals can cause organ mal-formations, disruption of organ function, and premature death. Furthermore functional maturity and expression of the various AR and DR in the vasculature of newborn vary greatly compared with those of older children and adults.[36,37]

With increasing clinical and animal data showing that hemodynamic responses to inotropes in newborns differ from those in adults and older children, the need for newborn studies becomes essential to understand the effect of commonly used inotropes in the NICU. The relative maturation rates of physiologic systems probably better understood in newborn animals than human newborn, as studies of drug effect on the neonate are difficult.

Systemic and regional responses of epinephrine and dopamine during hypoxia

Cheung et al compared the effect of dopamine (2,10, 32 mcg/kg/min) and epinephrine (0.2, 1.0, 3.2 mcg/kg/min) on the systemic, pulmonary, hepatic and mesenteric circulations in 17 anesthetized and instrumented hypoxic newborn piglets (PaO_2 40–50 mmHg, arterial saturation 55-65%),[38] They demonstrated that epinephrine was more effective than dopamine at increasing cardiac output during hypoxia, while in this model dopamine but not epinephrine, increased portal flow and total hepatic flow during hypoxia.

Barrington et al looked at the hemodynamic responses to dopamine and epinephrine infusions in newborn piglets during normoxia and hypoxia in a prospective randomized, blinded study of newborn piglets.[39] During normoxic epinephrine (0.2-1.6 mcg/kg/min) for 15 min improved mean arterial pressure, coronary artery flow pulmonary/systemic vascular resistance ratio compared to dopamine (2-16 mcg/kg/min). After hypoxia (arterial saturation of 45 to 50% for 20 min) and normoxia for 1 h reoxygenation, epinephrine (0.2-3.2 mcg/kg/min), at doses > 1.6 mcg /kg/min showed vasoactive effect with no effect on cardiac output, while at doses < 0.8 mcg/kg/min epinephrine significantly reduced pulmonary artery pressure. Dopamine (2-32 mcg/kg/min) had no effect on systemic, pulmonary blood pressure or the cardiac output but reduced systemic vascular resistance at high doses.

The effects of acute hypoxemia on cardiovascular responses to dopamine and dobutamine (2, 5, 15 mcg/kg/min) were studied in 2-4 day and 13-17 day old swine.[40] In newborn swine dopamine is a stronger inotropic agent than dobutamine during normoxemia and hypoxemia while the chronotropic effects of the two drugs did not differ. Changes in cardiac output with both drugs did not differ significantly during normoxemia and were not affected by hypoxemia.

Systemic and regional responses of epinephrine and dopamine during normoxia

McGovern et al studied the effect of dopamine, epinephrine and dobutamine on hemodynamic and energetic indices of the injured right ventricle and its interaction with the pulmonary vasculature in young piglets and showed that cardiac output significantly increased with each catecholamine infusion. Epinephrine decreased pulmonary artery pressure by 13% while dopamine increased PA pressure by 14% from the base-drug state. Pulmonary vascular resistance decreased by 36% with epinephrine and increased by 11% with dopamine. The conclusion was that epinephrine may be more advantageous to the acutely injured right ventricle.[41]

To determine the effects of a continuous epinephrine infusion on renal and mesenteric blood flow in both healthy and hypotensive newborn piglets, Bigam et al showed that epinephrine in doses more than 1.6 mcg/kg/min may cause vasoconstriction in the renal or mesenteric circulations during normovolemia or hypovolemia.[42]

Cheung et al investigated the response of the systemic, pulmonary, hepatic and portal circulations to infusion of dopamine (2, 10, 32 mcg/kg/min) and epinephrine (0.2, 1.0, 3.2 mcg/kg/min) in newborn piglets.[43] They showed that during normoxia higher doses of both drugs are needed to induce an increase in systemic, pulmonary blood pressure and cardiac output. Higher doses of epinephrine ≥3.2 mcg/kg/min were associated with systemic and regional vasoconstriction and increased plasma lactate.

The systemic, pulmonary and cardiac responses to epinephrine (0.2-3.2 mcg/kg/min) were studied in 5-10 days old piglets,[44] and Barrington et al showed that systemic and pulmonary vasoconstriction were observed at doses higher than 1.6

mcg/kg/min, while coronary blood flow and myocardial oxygen extraction ratio improved as the epinephrine dose increased.

The effects of increasing dosages of epinephrine infusion (0.01-2 mcg) on intestinal oxygen supply were studied in adult pigs.[45] The authors showed that epinephrine increased jejunal microvascular blood flow and mucosal tissue oxygen supply at moderate to high dosages. The lactic acidosis that develops during infusion of increasing dosages of epinephrine is not related to the development of gastrointestinal hypoxia.

With the controversy over the potential risk of higher oxidative stress with the use of high oxygen for the resuscitation of newborns, it is crucial to understand how may this affect the functional maturity of the systemic and regional circulation and hence their response to inotropic agents. Supplemental oxygen is one of the most widely used therapies in the NICU. The effect of hyperoxemia and increased oxidant stress during resuscitation with 100% oxygen on the cardiovascular hemodynamics of these infants has not been studied in asphyxiated newborns. Newborns have a high concentration of hemoglobin which is predominantly composed of fetal hemoglobin. Fetal hemoglobin combines more rapidly with oxygen at low tension than does adult hemoglobin.[46] The oxygen partial pressure of the fetal arterial blood normally ranges from 25 to 30 mmHg.[47] Even though oxygen tension in fetal blood is only one-fifth to one-fourth that of adult blood, fetal arterial blood oxygen content and oxyhaemoglobin saturation are not much lower than those of an adult due to the presence of fetal hemoglobin. Hyperoxemia is usually associated with the use of high oxygen during resuscitation which may therefore increase the content of dissolved oxygen and may generate higher oxidative stress, increasing the risk of tissue damage. This may affect the maturation of AR.

There is a paucity of clinical studies investigating the systemic and regional effects of epinephrine and dopamine in shocked asphyxiated neonates especially with the use of high oxygen concentration for resuscitation. Most of the clinical studies reviewed above were performed in preterm infants and under normoxic condition. While the cause of hypotension is multifactorial, the definition of hypotension in the newborn population is still not clearly defined. Indeed various criteria have been used to define normal blood pressure or hypotension in these studies which made it difficult to compare the results.

In summary, oxygen, epinephrine and dopamine are commonly used to support of the sick neonate, but most of the pharmacological knowledge of these drugs has been extrapolated to the newborn from adult studies. Little information is available on the systemic and regional effect of epinephrine and dopamine in shocked asphyxiated neonates especially with the use of high oxygen concentration for resuscitation. Most of the clinical studies reviewed in this chapter were performed in preterm infants and under normoxic condition. Only one small study found that the use of low dose dopamine in asphyxiated children may be useful. Animal studies have shown that there are differences in the systemic and regional responses to epinephrine and dopamine during hypoxia and normoxia. With the current controversies over the use of room air versus 100% oxygen for the resuscitation of the asphyxiated neonate, the regional and hemodynamic responses to epinephrine and dopamine during resuscitation with both gases is still not known.

The effect of low dose dopamine on the renal circulation.

Goldberg et al were the first to demonstrate the renal effects of low-dose dopamine (< 5 mcg/kg/min) in 1963 in patients with congestive heart failure.[48] Hollenberg et al showed that infusion of dopamine in healthy animals and normal volunteers results in augmentation of renal blood flow and diuresis.[49] The diuretic effect of dopamine at low doses is attributed to the effect of DR1 on the renal vasculature causing vasodilatation and diuresis as a result of the inhibition of Na+/K+ ATPase at the tubular epithelial cell level, which increases sodium excretion and, hence, diuresis.[50,51] Although low-dose dopamine has been shown to increase renal blood flow as well as promote diuresis and natriuresis, its role in the more important clinical measures including mortality, need for hemodialysis and biochemical evidence of organ function (serum creatinine or creatinine clearance) is not well established.[52,53] Systematic reviews of the effects of low dose dopamine on renal function in the critically ill adult showed no evidence that dopamine can prevent mortality, onset of acute renal failure or the need of dialysis.[54]

Prins at al looked at the current use of low-dose dopamine (<5 mcg/kg/min) to improve renal function and urine volume in the neonatal and pediatric ICU by assessing the available evidence to support this practice. The literature review identified seven clinical studies focused on preterm neonates, critically ill infants and children, and those who underwent cardiac surgery. Renal function improvement was measured by creatinine clearance, glomerular filtration rate and increase in urinary volume. Only one study was a randomized controlled trial in preterm neonates which showed no positive correlation between renal function and urinary volume. The conclusion is that the evidence from well performed clinical studies to support the use of low-dose dopamine for improving renal function and urinary volume in critically ill neonates and children is largely insufficient.[55]

In the Cochrane library, a systemic review by Barrington and Brion examined whether dopamine therapy can prevent indomethacin-mediated deterioration in renal function in the preterm newborn infant without serious adverse effects, and concluded that there is no evidence from randomized trials to support the use of dopamine to prevent renal dysfunction in indomethacin-treated preterm infants.[56]

On the other hand Lynch et al showed that dopamine infusion at a rate of 2.5 mcg/kg/min in normotensive, oliguric premature neonates (34±2 weeks) with respiratory distress syndrome significantly increased glomerular filtration rate and urine output, with no changes in blood pressure or heart rate.[57] Seri et al demonstrate a selective renal vascular response to low dose dopamine (6.1 ± 3.0 mcg/kg/min) in 2 day old, nonhypotensive preterm infants, [28] which was not observed in the mesenteric or the cerebral circulation. The authors reported that dopamine significantly increased blood pressure and urine output, but the increase in renal blood flow was independent of the blood pressure changes.

As seen above most of the neonatal studies were done in normoxic sick premature and term infants. The effect of low dose dopamine and epinephrine in asphyxiated newborns was not studied. Furthermore there is no reliable evidence that dopaminergic vasodilatation occurs in the neonatal mammalian or human renal circulation.[58] Indeed there is little evidence that dopamine improves either renal perfusion or renal function in the newborn. Animal studies investigating the renal effect of low dose dopamine in newborn studies showed poor response which was attributed to the immaturity of dopamine receptors in newborn animals.[59,60]

Low dose dopamine is not without side effects; it can increase the cardiac output and systemic vascular resistance which not always a desirable effect. [61] There have been reports of low dose dopamine causing tissue necrosis and digital gangrene.[62,63] It can increase myocardial oxygen consumption and cause myocardial ischemia and cardiac arrhythmias.[53]

In conclusion, low-dose dopamine may facilitate urine output and natriuresis but the overall benefit of this effect in renal protection is not evident from the clinical and animal studies. There is little evidence that dopamine improves either renal perfusion or renal function in newborns. The immaturity of dopamine receptors seen in newborn animal studies may be the reason for the poor renal response seen in the human newborn. Moreover, low dose dopamine use can be associated with serious side effects. The effect of low dose dopamine on renal flow during hypoxia-reoxygenation has not been studied in neonates.

4-5. Antioxidant and proxidant properties of catecholamines.

Catecholamines such as epinephrine and dopamine are widely used for the treatment of shock in asphyxiated infants as inotropic agents, but they can potentially function as antioxidants and proxidants.[64,65,66] These functions are mediated through AR and DR or through byproducts of oxidative metabolism.[67] Sofic et al looked at the antioxidant and proxidant capacity of catecholamine, using the oxygen radical absorbance which contain ROS generator assay. The antioxidant effect of catecholamines and related compounds were in the order: neurotransmitters: dopamine > norepinephrine > metabolites > amino acid precursors. The antioxidant effect of catecholamines and related compounds were linearly correlated with concentration.[68] While Miura showed that dopamine can inhibit lipid peroxidation, and scavenge $O_2^{\bullet-}$ and $OH^{\bullet}$ radicals more effectively than α-tocopherol, and much more effectively than norepinephrine or epinephrine.[64] The antioxidant function of epinephrine was reported by Hsu et al.[66] They showed that epinephrine ameliorated severe gastric hemorrhage and decreased gastric mucosal lipid peroxidation through α and β-AR and increased the levels of gastric mucosal NO and glutathione through modulation of α-AR in a rat model of gastric bleeding induced by lipopolysacchrides. While Mehta et al reported that epinephrine via β_1-AR activation causes $O_2^{\bullet-}$ anion generation, and the $O_2^{\bullet-}$ subsequently upregulated the endogenous antioxidant species SOD in human coronary artery endothelial cells.[69] Amin et al reported that α_1-adrenergic receptor activation by norepinephrine and ROS could induce hypertrophy in adult rat ventricular myocytes through the activation of signal- regulated kinases.[68]

The above studies indicate that proxidant and antioxidant functions of catecholamines can be mediated through AR activation. Other proposed mechanisms for the proxidant role of catecholamines include the formation of toxic byproducts. Catecholamines are metabolized by specific enzymes (Fig 4-1). Dopamine, norepinephrine and epinephrine can be deaminated by monoamine oxidase. This results in the formation of an aldehyde intermediate, which is oxidized further to an acid, or reduced to an alcohol. The toxic byproduct of this reaction is H_2O_2.[70] Catechol-o-methyl transferase catalyzes the methylation of one of the hydroxyl groups on the catechol ring by acting directly on the parent compound, but this can also occur after deamination by monoamine oxidase. The oxidative metabolism of catecholamines is an alternative metabolic fate. Under physiological conditions catecholamines can be non-enzymatically oxidized by molecular oxygen to form the corresponding *o*-quinone or aminochrome. The aminochromes undergoes an intramolecular cyclization which is immediately followed by a cascade of oxidative reactions resulting in the final formation of neuromelanin.[71] Reactive oxygen species are also produced in these processes. Toxic quinone metabolites of catecholamines may have a key function in the production of ROS with various pathological consequences, including apoptosis.[72] Glutathione is reported to have a role in the detoxification of ROS produced by catecholamines.[73] However the balance of the antioxidant and proxidant capacities of catecholamines is dictated by ambient conditions such as catecholamine concentration,[66] ROS concentration,[74] pH and oxygen content.[75]

Oxygen and catecholamines are commonly used to support asphyxiated infants. Reintroduction of oxygen can exaggerate the oxidative stress. The severity of the oxidative stress is dependent on the antioxidant availability. Because of the reported proxidant potential of the catecholamines, the effect of these drugs on the antioxidant reserve in the myocardial tissue during hypoxia and reoxygenation with 21 and 100% was studied in this thesis project.

Table 4-1: Adrenergic receptor classification, location and function

Adrenergic receptors (Subtypes)	Location and function
α_1 ($\alpha_{1A}, \alpha_{1B}, \alpha_{1C}, \alpha_{1D}$)	Vascular smooth muscles (vasoconstriction) Myocardium (positive inotrope) Bowel/bladder sphincters (contraction)
α_2 ($\alpha_{2A}, \alpha_{2B}, \alpha_{2C}$)	Vascular smooth muscles (vasoconstriction) Myocardium (chronotrope) Bowel smooth muscles (relaxation)
β_1	Myocardium (positive inotrope and chronotrope) Coronary artery smooth muscles (vasodilation) Skeletal smooth muscles (vasodilation) Kidney (renin release)
β_2	Bronchial smooth muscles (Bronchodilation) Vascular smooth muscles (vasodilation)
β_3	Regulation of fatty acid metabolism

Table 4-2: Tissue distribution of β adrenergic receptors.

Tissue	β_1-AR	β_2-AR
Heart	80%	20%
Kidney	70%	30%
Lung	30%	70%
Liver		≤100%
leucocytes	0%	100%

AR: adrenergic eceptors

Table 4-3: Dopamine receptors.

DR1	Smooth muscle relaxation Increase cAMP	Vasodilation of renal and splanchnic beds
DR2	Central nervous system Decrease cAMP	Inhibit prolactin, thyroid stimulating hormone, aldosterone

DR: dopamine receptors

Table 4-4: Cardiovascular effects of epinephrine at various infusion rates.[76]

0.02-0.2 mcg/kg/min β_1 and β_2 -AR	0.2-2 mcg/kg/min β and α-AR	Over 2 mcg/kg/min α-AR
Increased cardiac output and mild vasodilation	Increased cardiac output and systemic vascular resistance	Systemic and regional vasoconstriction and metabolic changes

AR: Adrenergic receptors

Table 4-5: Cardiovascular effects of dopamine at various infusion rates

	0.5 to 2 mcg/kg/min DA$_1$ receptor	2-10 mcg/kg/min β_1-AR	Over 10 mcg/kg/min α_1-AR
Cardiac output	No change	Increase	Increase
Stroke volume	No change	Increase	Increase
Heart rate	No change	There is an initial increase followed by a decrease toward normal rate as infusion continues.	
Myocardial contractility	No change	Increase	Increase
Potential for excessive myocardial oxygen demands	Low Coronary blood flow increased	Low Coronary blood flow increased	Data unavailable
Potential for tachyarrhythmia	Low	Low	Moderate
Total systemic vascular resistance	Slight decrease to no change	No change to slight increase	Increase
Renal blood flow	Increase	Increase	Decrease
Urine output	Increase	Increase	Decrease

DA: Dopamine receptor. AR: Adrengeric receptor

Table 4-6: Pharmacokinetics of epinephrine and dopamine.

	Epinephrine	Dopamine
Chemical formulae	$C_9H_{13}NO_3$	$C_8H_{11}NO_{22}$
Dose in NICU	0.05 -1.0 mcg/kg/min	2-20 mcg/kg/min
Mechanism of action	Direct sympathomimetic	Direct sympathomimetic and dopaminergic. Indirect release of norepinephrine
Protein bound	50%	unknown
$t_{1/2}$	5-10 minutes	2 minutes
onset of action	Instant	5 minutes
Peak	20 minutes	
Duration of action	20-30 minutes	less than 10 minutes
% of IV dose metabolized	90%	75%, (25%→norepinehrine)
Excretion products	VMA, MHPG	DOPAC, HVA
Inactivation	MAO-B, COMT	MAO-B, COMT, dopamine transporters

MAO: monoamine oxidase. COMT: catechol-o-methyltransferase. DOPAC: 3, 4-Dihydroxyphenylacetic acid. HVA: homovanillic acid. VMA:Vanillylmandelic acid. MHPG: 3-methoxy-4-hydroxyphenylglycol

Table 4-7: Afferent inputs in cardiovascular control

Afferent input		Location
Chemoreceptors	Central	Medulla
	Peripheral	carotid and aortic bodies
Mechanoreceptors (Baroreceptors)	Cardiopulmonary (low-pressure receptors)	Cardiac atria and ventricular myocardium Junction of the great veins Pulmonary vessels
	Arterial baroreceptors	Walls of the carotid sinus, aortic arch, great vessels and the heart

Fig 4-1: Adrenergic receptor (AR) activation and signal transduction mechanisms. The activation of α_2AR and β-AR involve the inhibition (G_i) and the activation (G_s) of adenylyl cyclase respectively. The changed level of second messenger cyclic AMP (cAMP) will initiate the cellular response. The activated α_1 receptors bind to $G_{\alpha q}$ protein, that activates phospholipase C (PLC) which generates inositol triphosphate (IP3) and diacylglycerol (DG) as second messengers by activating the phosphorylation of the membrane lipid phosphatidylinositol bisphosphate (PIP2) to initiate activation of protein kinase leading to a sharp and transient rise in intra- cellular calcium respectively.

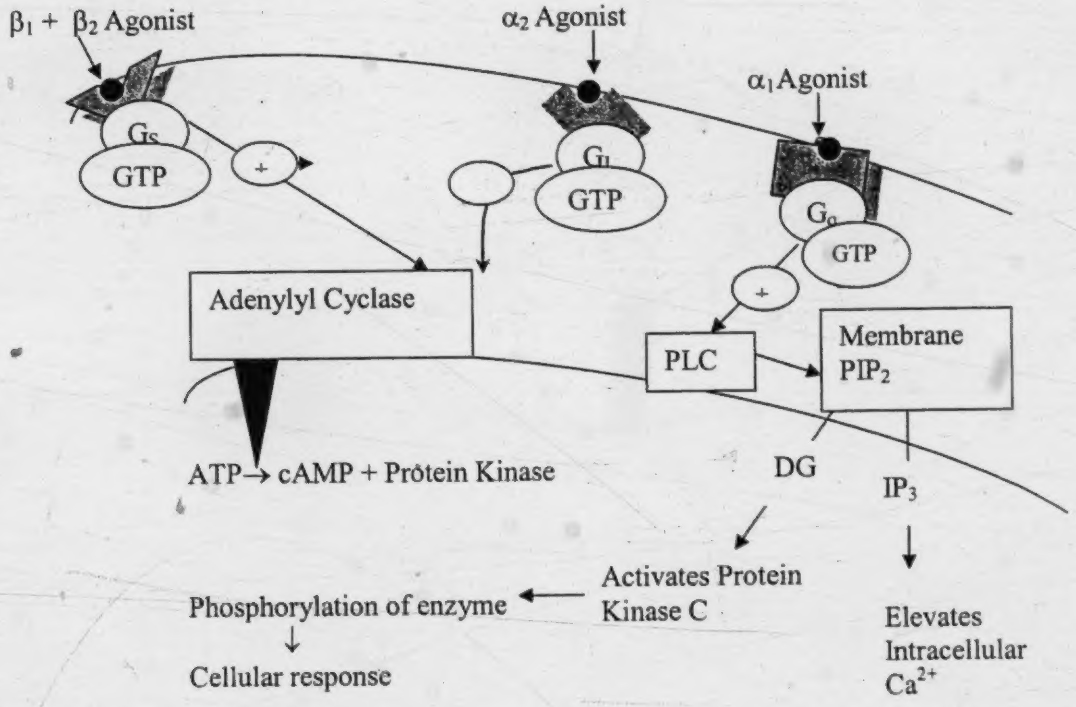

β_1 + β_2 Agonist
α_2 Agonist
α_1Agonist
Gs
GTP
+
Gi
GTP
Gq
GTP
+
Adenylyl Cyclase
PLC
Membrane PIP$_2$
ATP→ cAMP + Protein Kinase
DG
IP$_3$
Activates Protein Kinase C
Phosphorylation of enzyme
Cellular response
Elevates Intracellular Ca^{2+}

Fig 4-2: Epinephrine and dopamine synthesis and metabolism.

L-Tyrosine

↓ Tyrosine hydroxylase

L-DOPA

↓ DOPA Decarboxylase

Dopamine → MAO and COMT → DOPAC and HVA

↓ Dopamine B Hydroxylase

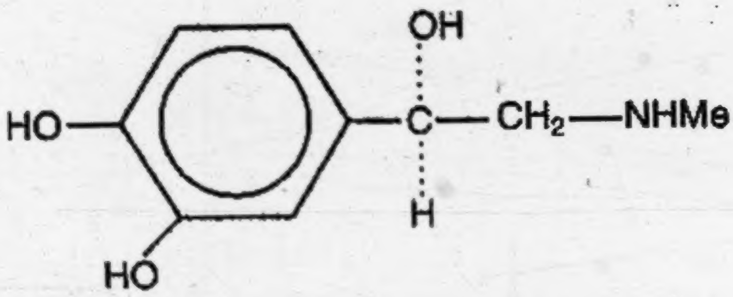
Noradrenaline

↓ Phenylethanolamine-N-methyl transferase

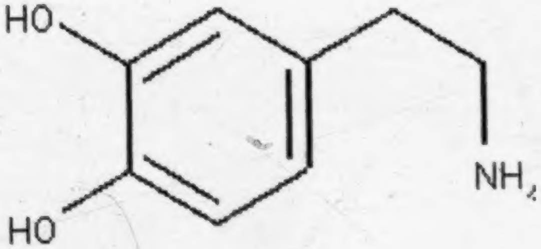
Adrenaline→ MAO and COMT→ VMA and MHPG

MAO: monoamine oxidase. COMT: catechol-o-methyltransferase. DOPAC: 3, 4-Dihydroxyphenylacetic acid. HVA: homovanillic acid. VMA:Vanillylmandelic acid. MHPG: 3-methoxy-4-hydroxyphenylglycol

Fig 4-3: The chemical structures of epinephrine and dopamine



Epinephrine (3, 4-dihydroxyphenyl)-2-methylaminoethanol



Dopamine $C_8 H_{11} NO_2$

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Chapter 5

The research project:

Newborn piglet models in neonatal research

5-1. Introduction

According to the American Academy of Pediatrics, newborn infants are much more likely to suffer from hypoxia and require resuscitation than any other age group. Perinatal asphyxia is a common clinical condition accruing in 3-5% of deliveries.[1] Ten million or more newborns worldwide each year need some type of resuscitation assistance. More than one million babies die annually from complications of birth asphyxia.[2] In Canada the neonatal death rate is 3.9 per 1000 live births, many of which are related to perinatal asphyxia.[3]

Perinatal and neonatal asphyxia may result in fetal demise, neonatal death, or a period of recovery during which there is cardio-pulmonary vascular dysfunction, organ failure and possible long-term effects, particularly in neurological function. There are many complications seen in infants following resuscitation. Neurological complication may manifest acutely as seizures or different stages of hypoxic ischemic encephalopathy. Long term neurological complications include cerebral palsy and major cognitive impairment. Cardiovascular manifestations include hypotension and poor ventricular function. PHT, acute tubular necrosis, NEC and liver dysfunction are other serious complication.[2]

There is increasing evidence that the total extent of hypoxic-ischemic insult is not completed within a period of minute or even an hour or two. Destructive processes after reoxygenation continue over hours and days.[4] This may give a therapeutic window during which we may be able to intervene to reduce the full extent of the hypoxia-reoxygenation damage.[5]

The injury of asphyxia to neonate systems seems to be mediated through a cascade of ROS generation during hypoxia- reperfusion, which can be markedly toxic to living tissues.[4] Following asphyxia, it is customary to resuscitate the newborn using a high oxygen concentration;[6] the use of 100% oxygen may result in increased generation of reactive oxygen species up to 4 weeks after birth.[4,7] and may impair cerebral blood flow at least in animal models.[8] Over the past decade there have been some literature from animal [9]and clinical studies[10,11,12] suggesting that resuscitation with room air is as efficacious as resuscitation with 100% oxygen for the short term outcomes, but the clinical significance of the biochemical findings associated with 100% oxygen resuscitation of newborn infants is still not clear and there are insufficient data to establish harm. This lead to the continuation to recommend the use of 100% oxygen for the resuscitation of newborns by the international guidelines conference on cardiopulmonary resuscitation and emergency cardiac care still recommend the use of 100% oxygen for neonatal resuscitation.[6]

Circulatory compromise with systemic hypotension is a relatively common complication of neonatal asphyxia in the term and preterm infants. The management of severe cardiovascular disorders is greatly facilitated when the patient's hemodynamic status can be quantitatively assessed. Blood pressure is one of the key parameters used by neonatologists to determine the hemodynamic status of the asphyxiated neonate. Blood pressure is the product of blood flow and vascular resistance (Ohms' law). So if the vascular resistance is high, it is possible for blood pressure to be high normally and flow to be low. If vascular resistance is low, pressure can be low and flow normal. Using blood pressure measurement for adequate perfusion may not be enough. Furthermore recent data highlighted the limitation of the use of blood pressure as the sole marker of circulatory failure.[13] The key to successful neonatal resuscitation is establishment of adequate ventilation, and adequate assessment of tissue perfusion would need the use of invasive procedures to measure oxygen delivery and consumption. Ultrasonography can be used to measure organ flow but the controversy over the best index to measure blood flow in different organ and the need for special training have limited it use in this field to research. Simple measurements such as mixed venous oxygen saturation, blood lactate level can give valuable information about the success of the resuscitation process.

Inotropes are introduced for the management of neonatal cardiovascular compromise associated with asphyxia in an attempt to improve outcomes in preterm and term neonates presenting with hypotension. Commonly used inotropes agents such dopamine and epinephrine[14,15] were introduced in neonatal intensive care without appropriately designed clinical trials. The choice of inotropes is dependent on many factors; the underlying pathophysiologic process, patient-specific responses to the insult, the relevant receptor expression and the downregulation of these receptors in critical illness in the systemic and regional circulation may influence the cardiovascular response to inotropes. The effect of the increased generation of ROS during 21% and 100% oxygen resuscitation on the respond oxygen on these factors is not clear and may influence the choice of inotropes

In summary, there are many complications seen in asphyxiated infants following resuscitation. The injury of asphyxia to neonate systems seems to be mediated through a cascade of biochemical reactions which include the generation of ROS hypoxia-reperfusion. To minimize the adverse effect of neonatal asphyxia we need to address the effects of commonly used drugs such as oxygen, epinephrine and dopamine on the systems of the developing neonate in the presence of hypoxia-reoxygenation injury, and the effect of oxygen concentration on the systemic and regional hemodynamics response to these inotropes in the presence of different degree of oxidant stress.

5-2. Newborn piglets in neonatal research

There are many ethical questions concerning research on newborn infants. Therefore, research is being done on newborn lambs, monkeys, pigs, mice, and rats.[16,17] Depending on the research question, certain animal models have been used more extensively in some research fields, for example newborn pigs models are widely used in cardiovascular and perinatal research.[18,19] Neonatal animal studies can be used to demonstrate increased or decreased susceptibility to adverse effects observed in mature animals and to understand the hemodynamic effect of drugs. Roohey et al evaluated 292 animal studies related to perinatal hypoxic ischemic encephalopathy.[20] They found rodents were the most frequently used animals in hypoxic ischemic encephalopathy research (26%), followed by piglets (23%) and sheep (22%). Asphyxia with or without ischemia was the most predominant method of producing experimental brain damage. The fetal sheep, newborn lamb and piglet models are well suited for the study of acute and subacute metabolic and physiologic endpoints whereas the rodent and primate models can be used for long-term neurological and behavioral outcome experiments.

Swine exhibits many similarities to man in cardiovascular anatomy and physiology including similarities in size and distribution of blood vessels, systemic and regional hemodynamics.[21,22]

The newborn piglet has been described as the best physiologic model among common laboratory species for comparison to human infants.[19,23] They are comparable in the degree of maturity, energy metabolism, and vascular anatomy to human newborns at birth.[24] The postnatal development of cardiovascular regulation in newborn piglets has been studied and it has been shown that they have very similar cardiopulmonary function, and pulmonary mechanics to human nfants.[25] They also have similar cardiovascular responses to common stresses in the human neonatal period, such as hypoxia, hypercapnia and hemorrhage.[26] Other advantages to using swine as an experimental model include: relatively low cost, availability, and the ability of swine to produce large litters of newborns, which are of suitable size for continuous hemodynamic monitoring during the study period.[18]

The pulmonary vasculature of newborn piglet is similar structurally to that of the human at birth, and although newborn piglet growth is more rapid, the pulmonary vasculature is remodeled in a similar fashion in the postnatal period to that of the human newborn. The adaptation of the pulmonary circulation to extrauterine life is not very different from what is observed in human newborns. The ductus arteriosus is anatomically open in piglets at birth but is reported to be functionally closed within 4 h of postnatal life.[27] Saugstad et al performed an echocardiographic examination in four piglets during hypoxemia and reoxygenation and found no flow in the ductus arteriosus in any of the piglets.[28] This result was also supported by other studies[29, 30] and observation in our laboratory (Dr. E Haase, personal communication). The foramen ovale is functionally closed at birth because of a higher pressure in the left side of the heart after birth.[21] The pulmonary hemodynamics at rest and under anesthesia are similar in piglets and humans.[19,31] Furthermore, piglets develop potent pulmonary vasoconstriction in response to alveolar hypoxia in a characteristic fashion as seen in newborn humans.[21]

Swine have multilobar kidneys similar in size, number, and structure to human kidneys; they have similar renal physiology and fetal and neonatal development as in humans.[32]

The gastrointestinal tract is very similar physiologically and anatomically to humans. The mesenteric vasculature response to cardiogenic shock has been studied in newborn piglets.[33] Interestingly, unlike other animal models, severe intestinal hypoperfusion does not cause septicemia secondary to bacterial translocation in the bowel.

The systemic and regional responses to catecholamines such as epinephrine and dopamine have been studied in the newborn piglet during normoxia and hypoxia,[34,35] and the presence of adrenergic[36,37] and dopamine receptors [38,39,40] in the systemic and regional vasculature has been established. The effect of low dose dopamine on renal perfusion during normoxia has also been studied and it was found that low dose dopamine infusion does not increase renal blood flow.[14]

In brief the newborn piglet model has been used extensively in perinatal and neonatal research, and it has been established that this species has similar cardiovascular anatomy and physiology to human. The postnatal development of cardiovascular regulation and the response to common perinatal stresses at birth is also similar to that reported in human newborn. Furthermore the systemic and regional response to commonly used catecholamines in the newborn period has been studied during normoxia and hypoxia. Therefore we adopted an established model of neonatal hypoxia and reoxygenation using newborn piglets to investigate the research questions.

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Chapter 6

General objectives and hypotheses

Oxidative stress is a condition in which oxidant metabolites exert their toxic effect because of an increased production or an altered cellular mechanism of protection, which occurs, for instance, during posthypoxic reoxygenation. Hypoxia causes alterations in the defense mechanisms against ROS, mainly a reduction in the activity of mitochondrial superoxide dismutase and a decrease of tissue content of reduced glutathione. At the same time, production of ROS increases in the mitochondria and leukocytes and toxic oxygen metabolite production is exacerbated by re-admission of oxygen during reoxygenation. Oxidative stress, in turn, causes oxidation of thiol groups and lipid peroxidation leading first to reversible damage, and eventually to necrosis.

Neonatal asphyxia remains an important condition with significant morbidity and mortality. Reoxygenation after severe hypoxia may contribute substantially to neonatal asphyxia-related organs injury and increased oxidative stress markers has been shown in the cord and plasma blood of asphyxiated infants. Myocardial injury and hemodynamic changes are commonly complicate neonatal asphyxia and can be associated with shock and low regional perfusion. Ischemic encephalopathy, pulmonary hypertension, necrotizing enterocolitis and acute renal failure are not uncommon consequence of neonatal asphyxia. High oxygen concentration and catecholamines such as epinephrine and dopamine are the main treatments used to support these infants. There are paucity in clinical studies comparing the systemic and regional responses to epinephrine and dopamine in asphyxiated term infants. Animal studies has shown that when compared to dopamine epinephrine is more effective inotrope in the treatment of shock post hypoxia and reoxygenation injury with no systemic and regional adverse effects in doses below 2 mcg/kg/min.

Furthermore the use of low dose dopamine to improve renal perfusion complicating neonatal asphyxia are widely used in the neonatal intensive care despite no clear evidence of the effectiveness of this treatment in the newborn infant and reported poor maturation of renal dopamine receptors .

Neonates may have reduced oxidative defenses due to the prematurity of the antioxidant systems and continuing developmental regulation of the antioxidant enzyme in the neonatal organs and plasma. Asphyxia and increased oxidative stress may disrupt the normal developmental secretion of active antioxidants and the depletion of existing antioxidant mechanisms. The antioxidant and proxidant dual nature of catecholamines has been reported. This proxidant effect is partly mediated through the production of ROS during the metabolism and autoxidation of catecholamines. The balance of the antioxidant and proxidant capacities of catecholamines is dictated by ambient conditions such as acidosis and oxygen content. The proxidant activity is thought to be directly proportional to the total number of free hydroxyl groups and epinephrine contains free hydroxyl groups more than dopamine.

The objectives of this study were to compare the systemic and regional hemodynamic responses of the newborn piglet to epinephrine and dopamine following severe asphyxia and resuscitation with 21% or 100% oxygen, and to examine the proxidant effect of both drugs in the myocardial tissue.

We hypothesized that:

1. Compared to dopamine, epinephrine is a more effective catecholamine for the treatment of hemodynamic failure in asphyxiated newborn piglets,

2. The use of 21% or 100% oxygen concentration in neonatal resuscitation can affect the systemic and regional responses to these drugs,
3. The low dose of either drug has no role in the support of the renal hypoperfusion associated with neonatal hypoxia, and
4. Epinephrine will induce more myocardial oxidative stress than dopamine in hypoxia-reoxygenation.

Chapter 7

The treatment of shock in asphyxiated newborn piglets resuscitated with 21% or 100% oxygen:

Epinephrine vs. dopamine.

Introduction

Asphyxia is a common clinical condition accruing in 3-5% of deliveries.[1] It can result in fetal demise, neonatal death, or a period of recovery during which there is cardio-pulmonary vascular dysfunction, organ failure and possible long-term effects, particularly in neurological function.[2,3]

Animal data have demonstrated that hypoxia, when preceding reoxygenation, leads to the augmentation of free radicals production (oxidative stress).[4] Following asphyxia, it is customary to resuscitate the newborn using 100% oxygen.[5] The use of 100% oxygen may result in the increased generation of reactive oxygen species (ROS) which are markedly toxic to living tissues.[6,7] A considerable literature from animal and clinical studies[8,9,10] suggests that resuscitation with room-air is as efficacious as resuscitation with 100% oxygen and that room-air resuscitation may limit the production of ROS.[4] Neonatal resuscitation guidelines recommend room-air for resuscitation only when positive pressure ventilation is needed and oxygen is unavailable.

Furthermore neonatal asphyxia is commonly associated with shock and low regional perfusion. Hemodynamic changes,[2] hypoxic ischemic encephalopathy, pulmonary hypertension, necrotizing enterocolitis and acute renal failure are not uncommon consequences of neonatal asphyxia.[3] Catecholamines such as epinephrine[11] and dopamine[12] are commonly used for the cardiovascular support of these infants when shock and hypotension develop following resuscitation. Blood pressure is only one factor that determines tissue oxygenation and recent data highlights the limitation of using blood pressure as the sole marker of improved systemic and regional perfusion.[11,13]

These agents act through specific receptors. The effects of oxygen concentration and ROS level on the regulation of these receptors during hypoxia and reoxygenation injury are not clear and may affect the sick infant's cardiovascular and regional responses to these agents. The systemic and regional responses to epinephrine and dopamine, including the use of low dose dopamine in the treatment of acute renal dysfunction, during 21% or 100% oxygen resuscitation have not been studied.

The objectives of the present study were to compare the systemic and regional hemodynamic responses of the newborn piglet treated with epinephrine or dopamine following severe asphyxia and resuscitation with 21% or 100% oxygen. We hypothesized that compared to dopamine, epinephrine is a more effective inotropic catecholamine for the treatment of hemodynamic failure during reoxygenation following severe hypoxia.

Methods

Animals

The study conformed to the regulations and approval of the Health Sciences Animal Policy and Welfare Committee of the University of Alberta. Forty two piglets (1-3 days old, weighing 1.4-2.6 kg) of Duroc breed were obtained on the same day of the experiment. Animals were block-randomized to 7 groups (n=6 each). A sham-operated group and six hypoxia groups; 2 control groups resuscitated with either 21% or 100% oxygen, and 4 treatment groups, resuscitated with 21% or 100% and treated with either epinephrine or dopamine, respectively.

Anesthesia

Anesthesia was induced with inhaled halothane 5% and maintained at 2% until a venous access was obtained and a tracheotomy with an insertion of endotracheal tube (3.5 mm, Portex Inc, Wilmington, MA) was done. Mechanical ventilation was commenced (Sechrist infant ventilator model IV-100, Sechrist Industries Inc. Anaheim, CA) with pressures of 18/4 cm H_2O at a rate of 18-20 breaths/min and inspired oxygen concentration of 21-25% to maintain transcutenous oxygen saturation between 89-95%, measured by a pulse oximeter (Nellcor, N-200, Nellcor Inc, City, Ohio). Once mechanically ventilated, halothane was discontinued; boluses of fentanyl (20 mcg) and pancuronium (0.6 mg) were given, followed by IV infusions of fentanyl (5-15 mcg/kg/hr), pancuronium (0.05-0.1 mg/kg/hr) and midazolam (0.1-0.2 mg/kg/hr). Additional boluses of fentanyl and pancuronium were given as needed.

Surgery

A single lumen arterial catheter (3.5F or 5F Argyle™, Sherwood Medical Co, St Louis, MO) was inserted into the distal aorta via the right femoral artery and was attached to a pressure transducer for continuous monitoring of mean systemic arterial blood pressure (SAP) and heart rate (HR). Venous access was obtained with 3.5F or 5-F Argyle double-lumen catheter inserted to the level of the right atrium via the right femoral vein for the administration of medications and intravenous dextrose–saline infusion (10 ml/kg/hr) and attached to a pressure transducer for continuous measurement of central venous pressure.

A left anterior thoracotomy was performed to open the pericardium and expose the main pulmonary artery. A 20G Insyte-W™ catheter (Becton Dickinson Infusion Therapy Systems Inc., Sandy, UT) was inserted into the root of the pulmonary artery for the measurement of mean pulmonary artery pressure (PAP) and mixed venous blood oxygen saturation. A six-millimetre transonic flow probe (6SB, Transonic Systems Inc., Ithaca, NY) was placed around the main pulmonary artery to measure cardiac output (CO). A 2-mm transit time ultrasound flow probe (2SS) was placed around the left common carotid artery (LCCA). Both probes were attached to a Transonic T206 animal blood flow meter for continuous measurement of blood flow.

Following the dose of acepromazine (0.25 mg/kg), the retroperitoneum was then opened via a left flank incision. The superior mesenteric artery (SMA) and left renal artery (LRA) were isolated and encircled with a 3-mm and a 2-mm transit time ultrasound flow probe (3SB and 2SB, respectively) attached to a Transonic blood flow meter for continuous measurement of blood flow.

Stabilization

The surgical procedure usually finished within 75 min. All incisions were closed or covered to minimize evaporative heat loss. The rectal temperature was maintained between 38.5-39.5°C by an electrical heating blanket and an over head warmer. After surgical manipulation all piglets were stabilized for at least 30 min. Stability was defined as (1) heart rate and blood pressure within 10% of the postanesthetic prethoracotomy values, (2) right atrial pressure of 3-8 mm Hg, (3) arterial PaO_2 75-100 mmHg, $PaCO_2$ 35 -55 mmHg, pH 7.35-7.45, and hemoglobin more than 65 g/l.

Blood samples were drawn for arterial, mixed venous oxygen saturation and hemoglobin concentration determinations by a co-oximeter OSM2 Hemoximeter, (Radiometer, Copenhagen). Analogue outputs of the pressure amplifiers and flow monitors were digitized by a DT 2801-A analogue to digital converter board (Data Translation, ON) in a Dell 425E personal computer. Software was custom written using the Asyst programming environment. All signals were continuously acquired at 24 Hz and saved on hard disk. These hemodynamic variables were monitored continuously for 6.5 h after stabilization in all groups.

Hypoxia-reoxygenation protocol

The sham-operated group underwent the experimental protocol only with no hypoxia-reoxygenation. In the hypoxic groups, normocapneic hypoxemia was induced by reducing FiO_2 to between 0.08 and 0.1 by increasing the concentration of inhaled nitrogen gas, until a drop in cardiac output of between 30 and 45% of baseline value was obtained. The duration of hypoxia was 45-60 min. Animals were randomized to reducing FiO_2 to between 0.08 and 0.1 by increasing the concentration of inhaled nitrogen gas, until a drop in cardiac output of between 30 and 45% of baseline value was obtained. The duration of hypoxia was 45-60 min. Animals were randomized to reoxygenation with either 21% or 100% oxygen for 1 h, followed by 21-25% oxygen for 4.5 h.

Catecholamine treatment protocol.

Before the infusion of catecholamine, all animals received 10 ml/kg saline solution to ensure sufficient intravascular fluid volume. No sodium bicarbonate was given and pretreatment pH was ≥ 7.25. At 2 h of reoxygenation animals were treated with either dopamine (2 mcg/kg/min) or epinephrine (0.2 mcg/kg/min) for 30 min (low dose infusion) in a double-blinded randomized fashion. The infusion rate was then gradually increased to maintain the SAP within 10% of baseline (optimal dose infusion) for a further 120 min.

Blood collection, hemodynamic measurements protocols

Blood samples were drawn and hemodynamic measurements were obtained at baseline, end of hypoxia, at 10 and 120 min of reoxygenation and 30 and 150 min of catecholamine infusion. Cardiac output, LCCA, SMA, LRA blood flows were indexed for piglet weight and expressed as ml/kg/min (CI, LCCAI, SMAI, LRAI, respectively)

At the conclusion of the protocol, the animal was euthanized using an overdose of IV pentobarbital (10 mg/kg).

Statistical analysis

Variables were analyzed by two-way repeated measures analysis of variance (ANOVA) followed by one-way ANOVA to determine differences within groups over time or between groups at each time point, respectively (Sigma Stat 2.0; Jandel Corporation, San Rafael, CA). ANOVA was carried out as above on ranks if tests of normality or equal variances failed. For post hoc pairwise comparisons, the Fisher Least Significant Difference method was used for one-way and repeated measures ANOVA, and Dunnett's method for ANOVA on ranks. Significance was defined as $P < 0.05$. The results are expressed as mean±standard error of mean.

Results

Hypoxia

Baseline hemodynamic parameters were not significantly different between groups (Table 7-1). The hemodynamic parameters remained stable during the course of experiment in the sham-operated group with no significant changes over time. Hypoxia (PaO_2 27±3 mmHg, SaO_2 27±4%) for a mean duration of 50 min, induced significant reduction in SAP (44±3% of baseline), CI (41±4%) and stroke volume index (SVI) (44±7%) (Fig 7-1 and Fig 7-2). There was metabolic acidosis (pH 7.07±0.03, bicarbonate 11±1 mmol/l) (Table 7-2). Systemic vascular resistance index (SVRI) decreased initially (76±8% of baseline) but normalized at end of hypoxia (95±10%).

Pulmonary arterial pressure and vascular index (PVRI) and PAP/SAP ratio increased significantly at the end of hypoxia (122±10%, 300±32%, 280±30% of respective baseline, respectively) (Fig 7-3 and Fig 7-4).

All regional blood flows dropped significantly with hypoxia (LCCAI: 79±9%, SMAI: 37±8%, LRAI: 36±10% of baseline). No significant differences were found in hemodynamic parameters at the end of hypoxia between all hypoxic groups.

Reoxygenation

Upon reoxygenation systemic and regional variables improved within 10 min of 21% or 100% reoxygenation except the elevated PAP, which normalized at 120 min of reoxygenation (Fig 7-4). The hemodynamic parameters deteriorated gradually over 2 h of reoxygenation reaching significantly lower values compared to baseline.

The recovery o f the systemic hemodynamics was different but not statistically significant between the 21% and 100% reoxygenation groups (Table 7-3). When compared to sham, the control 100% group had lower SAP, CI, stroke volume index, pH, and bicarbonate level with higher SVRI at the end of 4.5 h reoxygenation, whereas the differences between the control 100% and control 21% groups were modest (Table 7-3). The PAP was higher in the control resuscitated with 21% oxygen compared to control 100% and sham groups, but not the PVRI.

Catecholamine treatment

Animals in the hypoxic groups had normal acid base status (pH 7.32±0.03, bicarbonate 21±2 mmol/l) and central venous pressure (5±1 cm H_2O) prior to the start of catecholamine treatment with no significant differences between the groups.

Systemic hemodynamics

Low doses of both treatments (epinephrine at 0.2 mcg/kg/min, dopamine at 2 mcg/kg/min) for 30 min showed no effect on the systemic variables in both reoxygenation groups (Table 7-4).

Infusion rate needed to obtain the optimal dose in different animal was 0.5-2 mcg/kg/min for epinephrine and 10-25 mcg/kg/min for dopamine. Epinephrine at optimal dose for 2 h caused significant improvements in: SAP in both reoxygenation groups (epinephrine treatment in 21% reoxygenated group: 58±6 vs. 38±5 mmHg of controls; epinephrine treatment in 21% reoxygenated group: 58±5 vs. 34±4 mmHg of controls; both $p<0.005$) (Fig 7-1). There was increased CI with higher stroke volume in piglets resuscitated with 100% but not 21% oxygen (CI: 221±21 vs. 102±23 ml/kg/min of controls; p<0.01) (Fig 7-2). Dopamine at optimal dose for 2 h showed modest increases in SAP in both reoxygenation groups (41±2 and 44±4 mmHg at 21% and 100% groups, p= 0.06 and p<0.1 respectively) (Fig 7-1) with no significant effect on CI (Fig 7-2).

At optimal dose epinephrine significantly lowered PVRI and PAP/SAP ratio in the group resuscitated with 100% oxygen only (Table 7-4) (Fig 7-3). Dopamine significantly lowered the PAP/SAP ratio in the 100% group but not the PVRI.

Heart rate, PAP, systemic vascular resistances were not different between the groups at optimal doses of catecholamine treatment (Table 7-4) (Fig 7-4).

Regional arterial flow

At low dose epinephrine for 30 min significantly improved LCCAI and SMAI in the 100% reoxygenation group, while dopamine at low dose showed no significant effect on any of the regional (Table 7-5) (p<0.005).

In the 100% reoxygenation group epinephrine at optimal dose significantly improved LCCAI. Dopamine improved SMAI in the 100% reoxygenation group.

No significant effect was observed on LRAI in both reoxygenated groups at low and optimal dose (Table 7-5).

Oxygen metabolism

Systemic oxygen metabolism:

At optimal dose epinephrine for 2 h significantly improved systemic oxygen delivery (23±2 vs. 11±2 ml/kg/min of control, p<0.05) in the 100% reoxygenated group only. Dopamine showed no significant effect on systemic oxygen metabolism variables in both reoxygenated groups.

No significant differences observed in the systemic oxygen consumption and extraction between treatment groups at low and optimal doses (Table 7-6).

Regional oxygen metabolism:

At optimal dose epinephrine significantly improve LCA oxygen delivery in the 21% oxygen group. No other significant findings were observed on the regional oxygen delivery in both treatment groups (Table 7-7).

Discussion

Shock and hypotension are common complications of asphyxia in the newborn.[2] Asphyxia and reoxygenation can induce an oxidative stress.[4] Catecholamines such as dopamine and epinephrine were introduced for the management of neonatal cardiovascular compromise associated with asphyxia in an attempt to improve tissue perfusion.[11,12] Blood pressure is used clinically to assess response to catecholamines treatment, recent data highlighted the limitation of the use of blood pressure as the solely marker of improved systemic and regional perfusion.[11,13,14] Following asphyxia, it is recommended to resuscitate the newborn using a high oxygen concentration; the use of 100% compared to 21% oxygen may result in the increased generation of ROS, which are markedly toxic to living tissues.[7] Room air is advocated to be as efficacious as resuscitation with 100% oxygen and may limits the production ROS.[10,15,16]

The cardiopulmonary and regional response to epinephrine and dopamine following neonatal asphyxia and resuscitation with either 21% or 100% oxygen has not been studied.

Low dose epinephrine (0.2 mcg/kg/min) and dopamine (2 mcg/kg/min) for 30 min did not induce any significant systemic hemodynamic changes. Epinephrine at this infusion rate stimulates β-adrenergic receptors resulting in chronotropic and inotropic effects and systemic vasodilation.[17] In the current study epinephrine at low dose induced higher but not significant stroke volume index and lower SVRI and PVRI in the 100% but not the 21% reoxygenated group compared to the control and dopamine treated piglets. Dopamine receptors are selectively activated at low dose and pressure either does not change or decreases slightly.[18]

At optimal dose, although epinephrine (0.5-2 mcg/kg/min) for 2 h caused significant improvements in SAP in both 21% and 100% groups, higher CI, stroke volume index and lower SVRI, PVRI and PAP/SAP ratio was observed only in the 100% reoxygenated group only. Dopamine (10-25 mcg/kg/min) induced a modest increase in SAP and no significant effect on CI or the PVRI but PAP/SAP was significantly low in the 100% reoxygenated group. No significant hemodynamic changes noted with the optimal dose of dopamine in the 21% reoxygenated group. Heart rate and SVRI were not significantly different between the hypoxic groups.

Although the SVRI was not significantly different between the groups, the SVRI in the group resuscitated with 21% oxygen normalized at the end of reoxygenation in all hypoxic groups, while in the 100% reoxygenated groups only epinephrine maintained a lower SVRI than baseline value at optimal dose indicating no systemic vasoconstriction induced by epinephrine at dose up to 2 mcg/kg/min for 2 h in this model of severe hypoxia and reoxygenation of newborn piglet and the improvement in SAP was not mediated by systemic vasoconstriction. This was consistent with the finding of Cheung et al who showed significant elevation in SVRI and PVRI at epinephrine infusion rate at 3.2 mcg/kg/min, but not at 0.2, 1.0 mcg/kg/min, while dopamine at 2, 10, 32 mcg/kg/min had no significant changes on SVRI and PVRI.[19]

Despite no differences in PAP between the hypoxia groups in the current study, piglets in the epinephrine group had significantly lower PVRI and PAP/SAP ratio after reoxygenation with 100% compared to piglets in the control and dopamine groups. In the 21% reoxygenated group, the elevation in PVRI was 146% of baseline value in the epinephrine group and 177% in the dopamine group. The reported incidence of lung injury including pulmonary hypertension is up to 86% in post-asphyxiated newborns[3] and epinephrine at infusion rate up to 2 mcg/kg/min may has a more beneficial effect on pulmonary hemodynamics after 100% oxygen resuscitation and perhaps a less deleterious effect on the pulmonary hemodynamics after 21% oxygen resuscitation (Table 7-2).

The sick neonate is at increased risk of other complications such as necrotizing enterocolitis, intraventricular bleeding and renal failure.[3] The regional circulation response to different catecholamines at different infusion rates is an important factor in the choice of the catecholamines agent required for the treatment of shock in the asphyxiated neonate. This response depends on the functional maturity and expression of the various dopamine and adrenoceptors in the newborn. [20] The selective vascular response of regional circulation to dopamine and epinephrine has been reported in animal model of hypoxia-reoxygenation injury[19] and clinically.[21] To our knowledge the effect of 21% and 100% reoxygenation on the regional vascular response to these catecholamines has not been studied. In the current study both catecholamines showed differential effect on the regional flows; in the groups resuscitated with 100% epinephrine at low dose significantly improve SMA and LCCA flow while dopamine induced no regional flow changes including the renal artery flow, probably due to immaturity of the dopamine-1 receptor-mediated vasodilation mechanism. At optimal dose significant improvement was induced by epinephrine in the LCCA flow and by dopamine in the SMA flow in the 100% groups. No effect was observed of either drug at low dose on the renal artery flow.

In the treatment of shock in the asphyxiated infant, catecholamines are commonly used to improve cardiac output and tissue oxygen delivery. Our study showed that epinephrine (0.5-2 mcg/kg/min) significantly improved systemic blood pressure (perfusion pressure) in both resuscitation groups but systemic oxygen delivery was only significantly higher in the 100% group. Systemic oxygen consumption was also lower in all epinephrine groups, at both doses and resuscitation, compared to dopamine and control groups. Dopamine (10-25 mcg/kg/min) induces modest increase in SAP in the 100% group with no significant effect on systemic oxygen delivery in both reoxygenation groups. The regional oxygen delivery was not significantly different between groups, but there is a trend to increase with the increased regional flow described above (Table 7-5).

Reoxygenation with 100% oxygen may induce a higher level of hypoxia-reoxygenation injury. In the current study the control group reoxygenated with 100% had worse general status compared to the controls in the 21% reoxygenation group (control 100% vs. control 21%: CI 102 vs. 157 ml/kg/min, SAP: 34 vs. 38, pH: 7.25 vs. 7.30). Although epinephrine showed improvement of CI in the 21% group this was not significantly different from the less sick control in the 21% group which may explain the significant effective inotropic effect in the 100% but not the 21% reoxygenation group. The association between organ injury and ROS formation during hypoxia and reoxygenation process is well documented[22,23,24] and has been shown in clinical newborn studies that the immediate recovery after 21% reoxygenation can be better.[8]

We believe that 21% and 100% reoxygenation have different effects on the systemic and regional hemodynamics. Recent data from our lab showed higher CI measurement with 21% compared to 50% and 100% reoxygenation which were maintained for 30 min [25] and different mesenteric artery flow response to reoxygenation with 21%, 50% and 100% oxygen in severely hypoxic newborn piglets which correlated with the degree of oxidative stress.[26] ROS formations are now recognized as critical regulators of intracellular signaling cascades[27] and can stimulate the sympathetic nervous system.[28] The effect of the degree of oxidative stress generated during hypoxia and reoxygenation injury on the relevant receptor expression and the dysregulated release of local vasodilators and how this may affect the cardiovascular response to different inotropic agents is not fully understood and may explain the systemic response observed with epinephrine but not dopamine treatments in our model.

It is still unclear which catecholamines agent is appropriate in the treatment of shock or hypotension in sick newborns that are at risk for the development of persistent fetal circulation and necrotizing enterocolitis. Indeed, the appropriate catecholamine in various clinical situations also remains undetermined for these infants. With the current recommendation to use 100% oxygen for the resuscitation of these infants and the documented increased generation of ROS with higher oxygen concentration, the differential effect of the commonly used catecholamines such as epinephrine and dopamine on the cardiovascular and regional systems in severely asphyxiated newborn piglets resuscitated with 100 % oxygen is studied in the current experiment. In the current study we demonstrated that 100% reoxygenation may worsen the cardiogenic shock commonly seen in infant post asphyxia (CI: 171±30 vs. 140±28 ml/kg/min control 21% vs. control 100%). We also showed that epinephrine (0.05-0.2 mcg/kg/min) is an effective catecholamines in the treatment of shock in severely asphyxiated newborn piglets resuscitated with 100% oxygen and did not induce vasoconstriction in the pulmonary, carotid (carotid flow was improved significantly), renal or mesenteric circulation. At low dose (0.2 mcg/kg/min) epinephrine significantly improved mesenteric and carotid blood flow. Dopamine (10-25 mcg/kg/min) had a weak vasopressive action on the systemic and pulmonary vascular systems but significantly improver mesenteric flow. Dopamine showed no significant effects at low doses (2 mcg/kg/min) on the systemic and regional flow including the renal arterial flow.

Conclusions

During severe neonatal hypoxia followed by 100% resuscitation, epinephrine is more effective catecholamines than dopamine in the treatment of shock and hypotension, with a desirable effect on systemic oxygen delivery and carotid flow. Dopamine has a role in improving mesenteric blood flow. Caution should be applied when blood pressure alone is used a marker of tissue perfusion. Clinical and biochemical markers can be used in addition to blood pressure to assess systemic and regional responses to catecholamines treatment.

Table 7-1: Baseline hemodynamic parameters for the experimental groups.

	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH	CI (ml/kg/min)	SAP (mmHg)	PAP (mmHg)
Sham	75±3	39±1	7.34±0.02	201±21	62±2	26±2
Control 21%	73±3	39±2	7.35±0.02	222±25	64±3	26±1
Dopamine 21%	75±3	40±2	7.36±0.02	199±28	62±3	24±1
Epinephrine 21%	70±3	39±2	7.35±0.03	219±14	65±2	27±2
Control 100%	70±3	39±3	7.33±0.02	211±28	57±3	26±2
Dopamine 100%	70±2	39±2	7.37±0.02	224±30	64±3	25±3
Epinephrine 100%	75±3	41±2	7.36±0.02	234±17	62±3	24±3

21%: 21% reoxygenation. 100%: 100% reoxygenation. CI: cardiac index. SAP: systemic arterial pressure. PAP: pulmonary arterial pressure. PaO_2: arterial oxygen partial pressure. $PaCO_2$: arterial carbon dioxide partial pressure. Values are means±SEM

Table 7-2: Hemodynamic parameters for the experimental groups at the end of hypoxia.

	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH	CI (ml/kg/min)	SAP (mmHg)	PAP (mmHg)
Sham	73±4*	45±2	7.31±0.02*	165±17*	64±4*	26±1
Control 21%	29±2	42±3	7.07±0.04	70±10	28±4	32±3
Dopamine 21%	29±4	39±1	7.04±0.04	85±24	26±2	28±3
Epinephrine 21%	25±3	40±3	7.08±0.03	106±13	32±2	34±2
Control 100%	25±3	47±4	7.04±0.02	92±21	24±2	30±2
Dopamine 100%	28±3	38±1	7.09±0.04	96±14	28±3	30±3
Epinephrine 100%	28±2	38±3	7.08±0.04	102±11	27±4	31±2

21%: 21% reoxygenation. 100%: 100% reoxygenation. CI: cardiac index. SAP: systemic arterial pressure. PAP: pulmonary arterial pressure. PaO_2: arterial oxygen partial pressure. $PaCO_2$: arterial carbon dioxide partial pressure. $*p< 0.05$ sham vs. hypoxic groups. Values are means±SEM

Table 7-3: Hemodynamic parameters at end of 4.5 h of reoxygenation for the sham operated and the control groups reoxygenated with 21% oxygen (control 21%) and 100% oxygen (control 100%).

	Sham	Control 21%	Control 100%
pH	7.35±0.01	7.30±0.04	7.25±0.3
HCO_3 (mmol/L)	22±1	19±2	16±2
CI (ml/kg/min)	170±10	157±33	102±24
SAP (mmHg)	53±4	38±5*	34±4*
PAP (mmHg)	31±1	32±3	30±3
SVRI (mmHg.kg.min/ml)	0.33±0.03	0.23±0.02	0.39±0.09

PaO_2: arterial oxygen partial pressure. CI: cardiac index. SAP: systemic arterial pressure. PAP: pulmonary arterial pressure. *$p < 0.05$ vs. sham. Values are means±SEM

Table 7-4: Hemodynamic changes during low and optimal doses of epinephrine and dopamine compared to control.

	Baseline		Low dose infusion		Optimal dose infusion	
	21%	100%	21%	100%	21%	100%
HR (BPM)						
Control	204±12	214±19	227±11	237±10	249±15	245±8
Dopamine	210 ±9	197±15	250±13	234±18	260±10	252±10
Epinephrine	187 ±3	193±16	230±11	238±11	241±12	245±10
SVI (ml/kg)						
Control	1.1±0.08	1.1±0.20	0.8±0.12	0.6±0.11	0.7±0.2	0.5±0.08
Dopamine	1.0±0.12	1.1±0.20	0.6±0.09	0.8±0.16	0.6±0.08	0.6±0.16
Epinephrine	1.1±0.07	1.3±0.18	0.9±0.08	0.9±0.09	0.8±0.12	1.0±0.08*
SVRI‡						
Control	0.29±0.04	0.27±0.04	0.23±0.03	0.32±0.06	0.23±0.02	0.39±0.09
Dopamine	0.31±0.04	0.31±0.04	0.24±0.02	0.27±0.06	0.26±0.04	0.32±0.09
Epinephrine	0.29±0.04	0.25±0.04	0.25±0.02	0.19±0.03	0.28±0.03	0.24±0.02
PVRI‡						
Control	0.12±0.02	0.14±0.02	0.18±0.02	0.33±0.09	0.33±0.09	0.4±0.11
Dopamine	0.13±0.02	0.12±0.02	0.18±0.03	0.22±0.05	0.23±0.03	0.22±.04
Epinephrine	0.13±0.01	0.14±0.02	0.17±0.02	0.14±0.02	0.19±0.02	0.15±0.02*

SVRI: systemic vascular resistance index. SVI: stroke volume index. PVRI: pulmonary vascular resistance index. * $p < 0.05$ vs. control. ‡mmHg.kg.min/ml. Values are means±SEM

Table 7-5: Regional flow index at low and optimal doses of epinephrine and dopamine compared to control.

	Baseline		Low dose infusion		Optimal dose infusion	
	21%	100%	21%	100%	21%	100%
LCCAI[‡]						
Control	28±4	23±3	21±4	18±3	22±4	17±4
Dopamine	29±1	31±6	27±1	24±2	25±2	20±3
Epinephrine	31±4	28±2	31±4	28±3*	30±3	30±3*
SMAI[‡]						
Control	47±7	40±8	33±8	17±5	31±10	18±7
Dopamine	40±4	34±4	45±10	33±5	49±11	49±10*
Epinephrine	51±6	54±5	45±4	50±6†	40±40	40±8
LRAI[‡]						
Control	14±0.8	10±1.6	8.0±2.4	8.0±1.2	6.0±1.2	7.0±1.2
Dopamine	12±1.0	12±2.0	11±2.0	10±1.2	9.0±1.6	8.0±1.2
Epinephrine	12±1.6	12±0.8	12±1.2	10±0.8	9.0±1.2	8.0±1.6

SMAI: superior mesenteric artery index. LCCAI: left common carotid artery index. LRAI: left renal artery index. * P<0.05, †p<0.005, [‡] ml/kg/min. Values are means±SEM

Table 7-6: Systemic oxygen delivery (SDO$_2$) consumption (SVO$_2$) and extraction (SEO$_2$) at low and optimal doses of dopamine and epinephrine compared to respective control.

	Baseline		Low dose infusion		Optimal dose infusion	
	21%	100%	21%	100%	21%	100%
SDO$_2$‡						
Control	20±3	25±40	15±4	14±2	13±4	11±2
Dopamine	21±4	24±2	17±3	17±4	15±3	15±3
Epinephrine	22±2	26±2	19±2	22±2	20.±3	23±2*
SVO$_2$‡						
Control	7.2±1.0	6.8±1.1	6.2±0.9	5.1±0.8	5.9±0.5	4.4±1.2
Dopamine	4.8±1.0	5.4±0.9	4.8±1.1	5.1±0.9	4.2±0.7	4.8±0.7
Epinephrine	5.7±0.6	6.5±0.5	5.3±0.3	5.4±0.2	5.6±0.7	6.0±0.5
SEO$_2$						
Control	0.36±0.01	0.30±0.03	0.44±0.06	0.37±0.03	0.52±0.06	0.44±0.05
Dopamine	0.31±0.02	0.33±0.05	0.40±0.04	0.44±0.05	0.40±0.04	0.49±0.06
Epinephrine	0.34±0.02	0.37±0.04	0.40±0.05	0.37±0.03	0.42±0.06	0.40±0.06

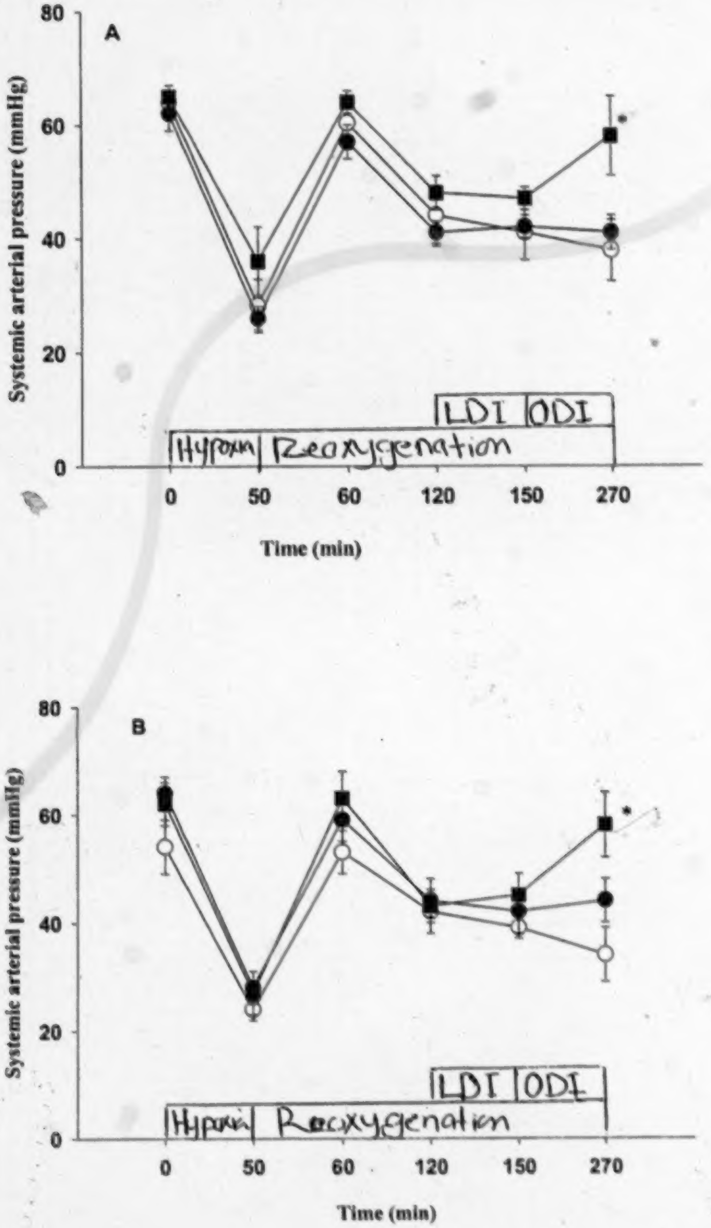
*$p<0.05$ vs. control. Values are means±SEM. ‡ ml/kg/min

Table 7-7: Regional oxygen delivery (DO$_2$) at low index at low and optimal doses of epinephrine and dopamine compared to control.

	Baseline		Low dose infusion		Optimal dose infusion	
	21%	100%	21%	100%	21%	100%
LCCA DO$_2$‡						
Control	2.7±0.4	2.8±0.4	1.9±0.5	2.3±0.2	1.9±0.4	1.9±0.4
Dopamine	3.0±0.2	3.5±0.9	2.7±0.1	2.5±0.4	2.4±0.1	2.0±0.5
Epinephrine	3.3±0.5	2.9±0.2	3.2±0.6	2.9±0.3	3.2±0.4*	2.8±0.3
SMA DO$_2$‡						
Control	4.2±0.6	5.0±1.4	2.5±0.8	2.5±1.0	2.3±0.88	3.0±1.7
Dopamine	3.6±0.8	3.9±0.5	4.0±1.2	3.5±0.7	4.2±1.3	4.1±1.3
Epinephrine	5.1±0.2	5.8±0.6	4.5±0.6	6.3±1.9	4.2±0.6	5.2±1.8
LRA DO$_2$‡						
Control	1.5±0.2	1.2±0.1	0.7±0.2	0.9±0.2	0.5±0.2	0.7±0.2
Dopamine	1.2±0.1	1.3±0.2	1.1±0.2	1.1±0.2	0.9±0.2	0.8±0.2
Epinephrine	1.3±0.2	1.1±0.2	1.2±0.1	0.9±0.1	1.0±0.2	0.8±0.2

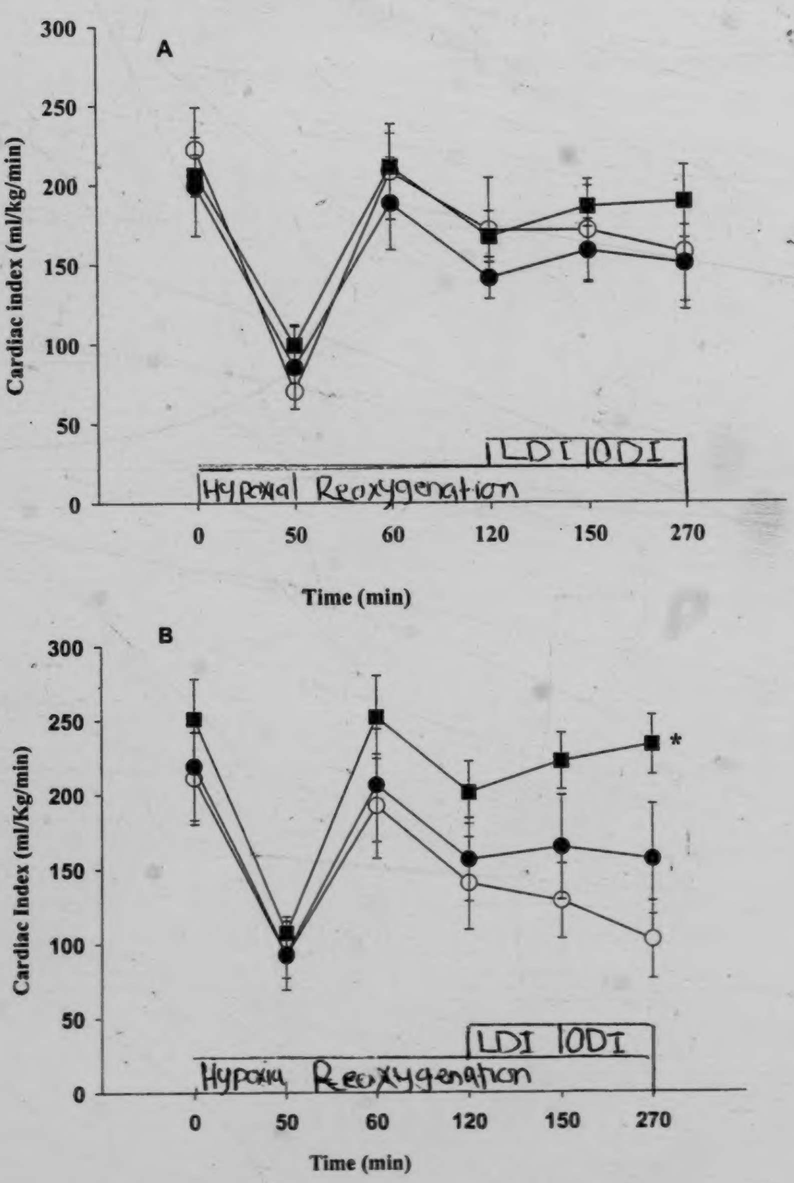
SMA DO$_2$:superior mesenteric artery DO$_2$. LCCA DO$_2$: left common carotid artery DO$_2$: LRA DO$_2$: Left renal artery DO$_2$. * P<0.05, ‡ ml/kg/min. Values are means±SEM

Fig 7-1: Systemic arterial pressure response to epinephrine (■) and dopamine (●) at low dose (LDI) and optimal dose (ODI) after hypoxia and reoxygenation with 21% (A) and 100% O_2 (B), compared to the control group (O).



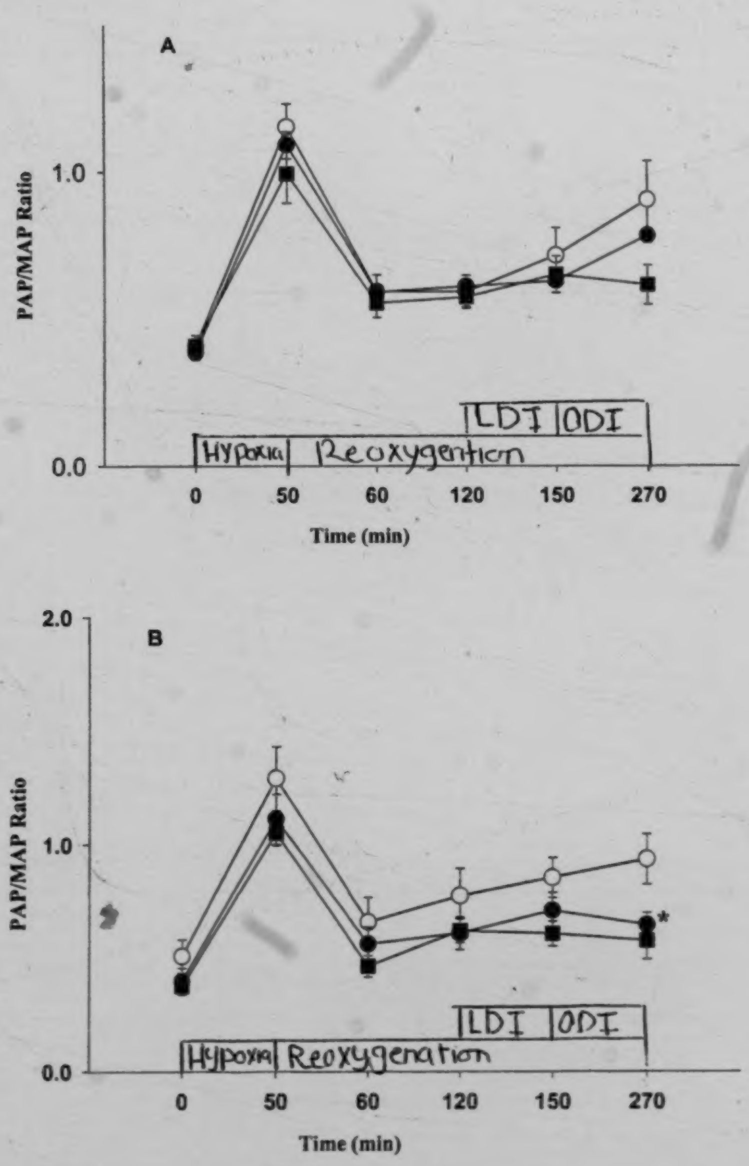
* $p < 0.05$ vs. corresponding controls. Values are mean±SEM.

Fig 7-2: Cardiac index response to epinephrine (■) and dopamine (●) at low dose (LDI) and optimal dose (ODI) after hypoxia and reoxygenation with 21% (A) and 100% O_2 (B), compared to the control group (O).



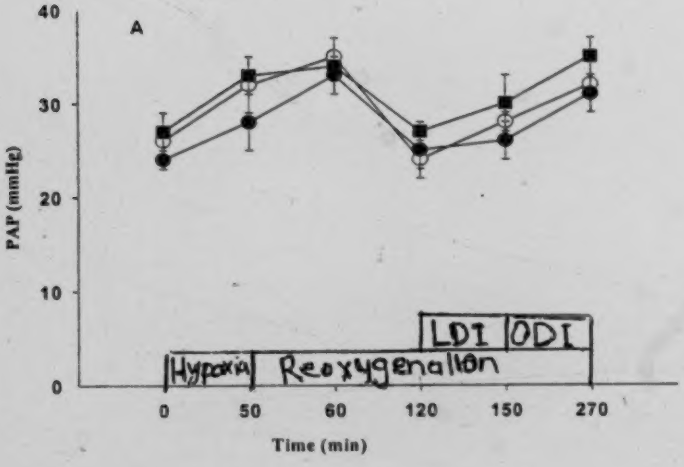
* $p < 0.05$ vs. corresponding controls. Values are mean±SEM.

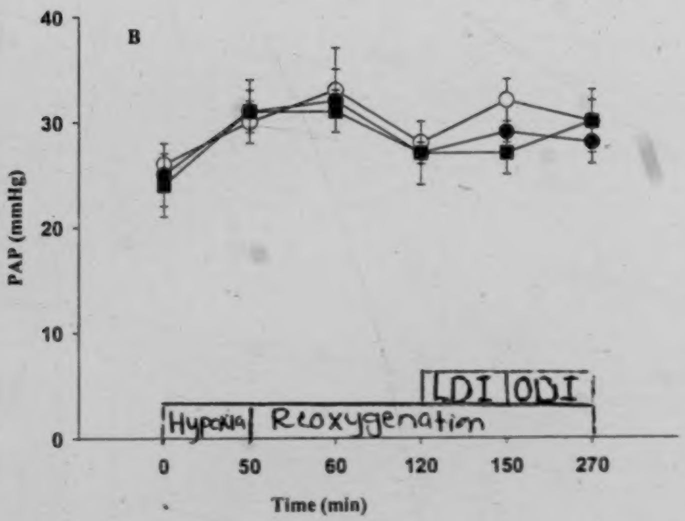
Fig 7-3: Pulmonary to systemic arterial pressure (PAP/SAP) ratio response to epinephrine (■) and dopamine (●) at low dose (LDI) and optimal dose (ODI) after hypoxia and reoxygenation with 21% (A) and 100% O_2 (B), compared to the control group (O).



* $p < 0.05$ vs. corresponding controls. Values are mean±SEM.

Fig 7-4: Pulmonary arterial pressure (PAP) response to epinephrine (■) and dopamine (●) at low dose (LDI) and optimal dose (ODI) after hypoxia and reoxygenation with room air (A) and 100% O_2 (B), compared to the control group (O).





* $p< 0.05$ vs. corresponding controls. Values are mean±SEM.

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Chapter 8

The effect of epinephrine and dopamine on the myocardial glutathione in asphyxiated newborn piglets resuscitated with 21% or 100% oxygen:

Epinephrine proxidative effects

Introduction

Oxidative stress mediated by reactive oxygen species (ROS) has been implicated in the pathogenesis of a wide spectrum of diseases in the newborn.[1] The severity of the oxidative stress depends on the balance between ROS production and removal by the antioxidant pathways. The newborn infant has a low level of antioxidant enzymes and scavengers which may be depleted during stress and use of oxygen.[2] Stress such as hypoxia and ischemia cause alterations in the defense mechanisms against ROS; mainly a reduction in the activity of mitochondrial superoxide dismutase and a depletion of tissue content of reduced glutathione (GSH).[3] Re-introduction of oxygen during reoxygenation and reperfusion may increases the production of ROS in the mitochondria and leukocytes.[4] Interestingly, the use of 100% oxygen in resuscitating asphyxiated neonates has been associated with markers of increased oxidative stress at 72 h and up to 28 days after birth compared with those in 21% oxygen resuscitation.[5]

This oxidative stress may cause oxidation of thiol groups and lipid peroxidation leading to reversible damage, apoptosis and/or cell necrosis in various organs,[6] particularly the heart. The neonatal myocardium responds to injury different from that of adult myocardium, due to different mechanisms of calcium influx and lower antioxidant capacity in the newborn heart.[7] Asphyxiated infants are at increased risk of myocardial injury, and up to 75% of these infants will manifest immediate hemodynamic changes.[8] The correlation between the extent of oxidative stress and myocardial cell damage has been studied extensively.[9,10] The oxidative stress can be quantified indirectly by the measurement peroxidation products and antioxidant levels like GSH and oxidized glutathione (GSSG).

Catecholamines such as epinephrine and dopamine are widely used for the treatment of shock in asphyxiated infants as inotropic agents, but they can potentially function as antioxidants and proxidant.[11,12,13,14] These functions are mediated through adrenergic and dopaminergic receptors or through byproducts of oxidative metabolism, aldehyde intermediates, hydrogen peroxide and *o*-quinone and aminochrome.[15,16, 17]

Because of the controversial proxidant role of catecholamines, we used a newborn piglet model of severe hypoxia and reoxygenation resuscitated with either 21% or 100% oxygen to examine the oxidative stress in the left ventricle when either epinephrine or dopamine was used to optimally support the hypotension developed post asphyxia. Since the proxidant activity is thought to be directly proportional to the total number of free hydroxyl groups and epinephrine contains more free hydroxyl groups than dopamine, we hypothesized that epinephrine would induce more oxidative stress, measured as higher oxidized glutathione, than dopamine in the myocardial tissue during 100% reoxygenation after hypoxia.

Methods

All experiments were conducted in accordance with the guidelines of, and approved by, the Health Sciences Animal Policy and Welfare Committee, University of Alberta.

Animal preparation and experiment protocol

The animals were prepared as detailed in chapter 7. Briefly, following hypoxia and stabilization, the animals were randomized to receive either 21% or 100% reoxygenation for 1h and then 21% oxygen until the end of the experiment. After 2 h of reoxygenation the animals were randomized to receive either epinephrine (0.2 mcg/kg/min) or dopamine (2 mcg/kg/min) for 30 min. The infusion rate was then gradually increased until SAP within 10% of baseline was reached (optimal dose) for a further 120 min, this was achieved with epinephrine in the range of 0.5-2 mcg/kg/min and dopamine of 10-25 mcg/kg/min.

Hemodynamic measures

Hemodynamic recordings for data analysis were carried out at specified time points: baseline, end of hypoxia and at 4.5 h reoxygenation (after 150 min of catecholamine infusion). Heart rate, mean systemic arterial blood pressure and cardiac output were continuously monitored.

Blood and tissue collection

Arterial blood gases analysis and hemoglobin measurements by a co-oximeter OSM2 Hemoximeter, (Radiometer, Copenhagen) were performed at specified time points as above.

Immediately after the piglets were euthanized (with 100 mg/kg IV pentobarbital), the heart was removed and a portion of the left ventricle wall was snap-frozen in liquid nitrogen and stored at -80°C until biochemical analysis.

Determination of myocardium reduced and oxidized glutathione in the left ventricule

Levels of GSH and GSSG in the left ventricular myocardium were determined using a glutathione assay kit (Cayman Chemical, Ann Arbor, MI). Briefly, 50 mg ±10% were homogenized in 500 μL of cold buffer consisting of 0.4 M 2- (N-morpholino) ethanesulphonic acid, 0.1 M phosphate, and 2 mM EDTA, pH 6.0 (MES). The sample was then centrifuged at 10,000 x *g* for 15 min at 4°C. The supernatant was removed and stored at -20°C. Deproteination of all samples was accomplished by adding an equal volume of 10% MPA (metaphosphoric acid) to the sample. The mixture was allowed to stand for 5 min, then centrifuged at >2,000 g for 5 min. The 4 M triethanolamine (TEAM) reagent was added to the samples (50 ul/ml of supernatant). The assay was performed by adding the assay cocktail [MES buffer, cofactor mixture, enzyme mixture, and 5,5'-dithiobis-2-nitrobenzoic acid (DTNB)] to the wells containing standards and samples. The microplate was incubated in the dark on an orbital shaker and the absorbance was measured at 25 min at 405 nm using a microplate reader (Titertek Multiscan PULS, MKII, Labsystems, Finland).

The total glutathione concentration was calculated using a standard curve. Oxidized glutathione (GGSG) was measured by mixing deproteinated samples with 1 M 2-vinylpyridine and incubating for 1h at room temperature. The assay then was performed as described above. Levels of GSH were determined by subtracting GSSG from the total glutathione levels. GSH and GSSG content were expressed as umol/ml protein. Ratio of GSSG: total glutathione was calculated. Tissue protein concentration was assayed using bicinchoninic acid protein assay kit (Sigma) with albumin as standard.

Statistical analysis

Results are expressed as mean ± SEM. The hemodynamic variables were analyzed by two-way repeated-measures analysis of variance (ANOVA) followed by one-way ANOVA to determine differences within groups over time or between groups at each time point, respectively (Sigma Stat 2.0; Jandel Corporation). ANOVA was carried out as above on ranks if tests of normality or equal variances failed. *Post hoc* testing using the Fisher LSD method for one-way and repeated measures ANOVA or Dunnett's method for ANOVA on ranks was used for pairwise comparisons. One-way ANOVA with Fisher Least Significant Difference was used to determine differences in myocardial glutathione levels. Significance was defined as $p < 0.05$.

Results

Baseline hemodynamic parameters, animal weight and age were not significantly different between groups (Table 8-1). The hemodynamic parameters remained stable during the course of the experiment in the sham operated group. Hypoxia (PaO_2 27±3 mmHg, SaO_2 27±3%) significantly reduced CI (42±2% of baseline) and SAP (44±8%), with no effect on heart rate. This was associated with metabolic acidosis (pH 7.06±0.08, bicarbonate 11±3 mmol/L). After an immediate recovery, cardiogenic shock gradually developed over 2 h in both reoxygenation groups. No significant systemic hemodynamic changed was observed at low doses of both drugs. Epinephrine significantly improved SAP in both 21% and 100% reoxygenation groups, but this was associated with improvement in CI only in the group resuscitated with 100% oxygen. Dopamine induced a modest increase in SAP with no effect on CI in both 21% and 100% reoxygenation groups (Table 8-1).

Left ventricular glutathione level

The left ventricular tissue total glutathione, GSH contents were not significantly different between the groups, although GSH was modestly higher in the sham compared to controls in both reoxygenated groups (Table 8-2). Left ventricular GSSG level and GSSG: total glutathione ratios were significantly higher in the epinephrine group resuscitated with 100% oxygen compared to respective control (Fig 8-1, 8-2). Interestingly these variables were also significantly higher in the epinephrine group resuscitated with 100% oxygen compared to all other experimental groups.

Discussion

Cardiovascular complications are commonly associated with neonatal asphyxia; up to 75% of these infants will manifest immediate hemodynamic changes, including hypotension and ventricular dysfunction.[8] Furthermore these infants are at an increased risk of myocardial injury[18] due to different mechanisms of calcium influx and lower antioxidant capacity in the newborn infant heart.[2] Controversy exists over the use of 21% versus 100% oxygen for the resuscitating of these infant to minimize the oxidative stress. Hypoxia/ischemia and the reoxygenation process are associated with oxidative stress. Oxidative stress and free radical formation has been implicated in tissue injury including myocardial injury during neonatal asphyxia[8] with 50% to 70% of ROS liberated during the first few minutes of reperfusion.[19] Evidence of increased oxidative stress up to 28 postnatal days following the use of 100% oxygen for the resuscitation of asphyxiated infants has been reported in clinical studies,[5] although, the long term effect of this finding is still not clear.

Epinephrine and dopamine are commonly used catecholamines in the cardiovascular support of the asphyxiated infants. Catecholamines are reported to have the dual nature of antioxidant and pro-oxidant functions.[12] Dopamine was shown to inhibit lipid peroxidation, scavenge superoxide and hydroxyl radicals more than α-tocopherol and much more than norepinephrine and epinepherine,[14] while epinephrine decreased gastric mucosal lipid peroxidation through α- and β- adrenoceptors and increased the levels of gastric mucosal nitric oxide and glutathione through modulation of α- receptors in rat model of gastric bleeding induced by lipopolysacchrides.[13] The antioxidant function of these catecholamines is probably mediated through adrenergic[13] and dopamine receptors[20] and by scavenging free radicals.[12] However the proxidant function is mediated through byproducts of catecholamine metabolism. There are 2 types of catecholamines metabolism; enzymatic metabolism by monoamine oxidase and catechol-o-methyl transferase and non enzymatic oxidative metabolism. Reactive oxygen species are produced by both mechanisms. Under physiological conditions catecholamines can undergo non-enzymatic oxidation by molecular oxygen to form the corresponding *o*-quinone or aminochrome. The aminochromes undergo an intramolecular cyclization which is immediately followed by a cascade of oxidative reactions resulting in the formation of neuromelanin[21] and the production of ROS. During normoxia condition these free radicals are neutralized by the body's antioxidant systems. One of the important antioxidant mechanisms is the glutathione system, which is found abundantly in the human body and is present in intracellular and extracellular compartments. However upon reoxygenation following hypoxia, excess production of ROS may deplete the antioxidant reserve in the tissue and increase the proxidant potential of catecholamines.

There is evidence that glutathione play a role in the protection against the proxidant effect of the catecholamines[22,23] and free radicals myocardial cells induced injury.[10] It appears that the balance between the antioxidant and proxidant capacities of catecholamine is dictated by ambient conditions such as catecholamine concentration,[13] ROS concentration,[24] pH and oxygen content.[25] Palumbo et al[21] reported that the oxidation of 6-nitrodopamine and 6-nitroepinephrine, neurocytotoxic metabolites of catecholamines, by free radicals is pH -dependent and can explain catecholamines neurotoxicity associated with acidosis and oxidative stress.

In this study we used an established model of severe hypoxia and reoxygenation in the newborn piglet to examine the left ventricular tissue oxidative stress during treatment with epinephrine and dopamine during 21% and 100% reoxygenation. Although there was no significant difference in the GSH level between the groups, animals in the sham and 21% group had apparently higher GSH. Although there was a trend to higher oxidative stress in hypoxic piglet reoxygenated with 100% (C100) than the 21% reoxygenation group (C21) and sham groups, the difference was not significant.

The increase in left ventricular tissue oxidative stress in the epinephrine group reoxygenated with 100% oxygen could be due to increased production of ROS and/or reduced antioxidant capacity. Increased generation of ROS during reoxygenation may induce further production of free radicals either by triggering a cascade of ROS generation or by stimulating adrenergic receptors. Furthermore, increased oxidative metabolism of epinephrine and the formation of cytotoxic byproducts for example nitroepinephrine, which may further deplete the antioxidant capacity of the myocardial cells. This may explain the absence of increased oxidant stress in the epinephrine group reoxygenated with 21% oxygen as the same dose of epinephrine used and the acidosis status of all groups were not significantly different.

In this study although epinephrine showed an effective inotrope effect in the treatment of shock in severely asphyxiated newborn piglets resuscitated with 100% oxygen compared to dopamine, this was associated with increased oxidative stress, and the clinical significance of this finding on the compromised myocardium of the asphyxiated newborn is still unknown. Our findings raise the concern of the prolonged administration of epinephrine (hours and days in clinical practice) on the oxidative stress on the newborn heart.

In conclusion, epinephrine is an effective inotrope compared to dopamine for the treatment of shock in asphyxiated infant reoxygenated with 100% oxygen but this was associated with an increased level of oxidant stress which was not found in the groups reoxygenated with 21%. Caution is required when epinephrine is used in the presence of hypoxia-reoxygenation injury and increased generation of free radicals.

Table 8-1: Hemodynamic variables at baseline, end of hypoxia and 4.5 h of reoxygenation.

	HR	SAP	CI	pH	PaO_2
Baseline					
Sham	185±13	62±3	170±22	7.34±0.02	75±3
C21	204±12	64±3	223±25	7.35±0.02	73±3
C100	214±19	57±3	211±28	7.33±0.02	70±3
D21	212±10	62±3	199±28	7.36±0.02	75±3
D100	197±15	64±3	224±30	7.37±0.03	70±2
E21	187±33	65±2	219±14	7.35±0.03	76±3
E100	193±16	62±4	234±17	7.36±0.02	75±3
Hypoxia					
Sham	200±17	64±4	165±17	7.31±0.02	75±3
C21	212±9	28±4	70±10	7.07±0.04	29±2
C100	184±15	24±2	92±21	7.04±0.02	25±3
D21	197±16	26±2	85±24	7.04±0.04	29±4
D100	186±12	28±3	96±14	7.09±0.04	28±3
E21	193±12	32±2	106±13	7.08±0.03	25±3
E100	192±15	27±4	102±11	7.08±0.04	28±2

	HR	SAP	CI	pH	PaO_2
End of reoxy					
Sham	242±12	53±4	162±4	7.34±0.01	75±3
C21	249±15	38±5	157±33	7.30±0.04	77±6
C100	203±25	34±4	102±24	7.25±0.03	51±7
D21	260±11	41±3	150±22	7.34±0.03	70±4
D100	265±11	44±4	159±32	7.28±0.06	69±6
E21	241±12	58±6*	201±22	7.28±0.02	71±3
E100	248±11	58±5*	221±21*	7.29±0.02	71±5

* $p<0.05$ treatment group vs. respective control group

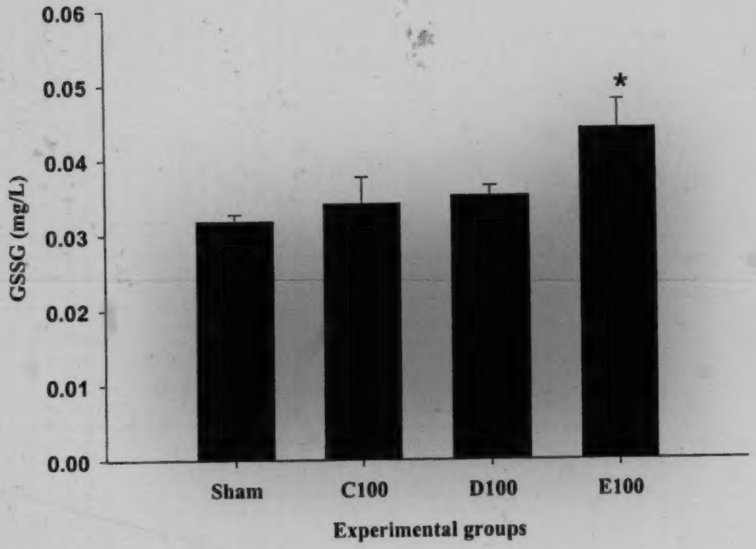
Heart rate (HR) BPM. Systemic arterl pressure (SAP) mmHg. Cardiac index (CI) ml/kg/min. Arterial partial pressure of oxygen (PaO_2). Hypoxic controls reoxygenated with 21% oxygen (C21) or 100% oxygen (C100). Hypoxic treatment groups reoxygenated with 21% or 100% oxygen and received either dopamine (D21) and (D100) respectively or treated with epinephrine (E21) and (E100) respectively. Values are mean±SEM.

Table 8-2: Cardiac Left ventricle glutathione components level in the experimental groups.

	TG (mg/L)	GSSG (mg/L)	GSSH/GSH Ratio	Reduced GSH (umol/L)
Sham	0.330±0.02	0.032±0.001	0.098±0.012	180±15
C21	0.348±0.02	0.032±0.002	0.093±0.002	179±13
C100	0.353±0.03	0.034±0.004	0.096±0.004	177±10
D21	0.353±0.04	0.033±0.004	0.095±0.003	182±25
D100	0.386±0.03	0.035±0.001	0.092±0.004	199±12
E21	0.354±0.02	0.035±0.002	0.098±0.003	184±16
E100	0.390±0.03	0.044±0.004*	0.113±0.006*	195±8.0

* $p < 0.05$ Treatment group vs. respective control. TG= total glutathione. GSH= reduced glutathione. GSSG= oxidized glutathione. Values are mean±SEM.

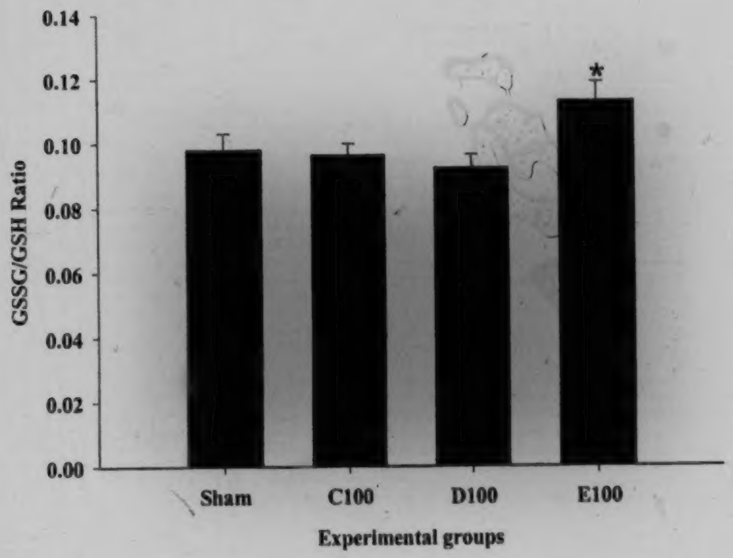
Fig 8-1: Cardiac left ventricular oxidized glutathione (GSSG) in the sham and groups received 100% oxygen.



* p<0.05 group vs. all groups (ANOVA).

Hypoxic controls reoxygenated with 100% oxygen (C100). Hypoxic treatment groups reoxygenated with 100% oxygen and received either dopamine (D100) or epinephrine (E100). Values are mean±SEM.

Fig 8-2: Cardiac left ventricular oxidized glutathione (GSSG) to total glutathione (GSSG/GSH) ratio in the sham and groups received 100% oxygen.



* $p<0.05$ group vs. E100.

Hypoxic controls reoxygenated with 100% oxygen (C100). Hypoxic treatment groups reoxygenated with 100% oxygen and received either dopamine (D100) or epinephrine (E100). Values are mean±SEM. Values are mean±SEM.

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Chapter 9

Conclusions and limitations

Approximately one of every sixteen newborns will require resuscitation of some kind in the delivery room. The aim of resuscitation is to prevent death and adverse long-term neurodevelopmental morbidity. The injury of asphyxia to neonatal systems is mediated in part through a cascade of reactive oxygen species generation during hypoxia and reoxygenation. There are increasing evidences of higher and prolonged oxidative stress associated with the use of 100% oxygen for resuscitation, the systemic and regional responses to commonly used catecholamines such as epinephrine and dopamine to treat shock and hypotension complicating neonatal asphyxia during 21% and 100% resuscitation has not been examined. We used an established newborn piglet model of acute hypoxia-reoxygenation injury to compare the effect of epinephrine and dopamine treatment on cardiopulmonary function, regional hemodynamics and the myocardial oxidative stress by measuring reduced and oxidized glutathione in left ventricular tissue obtained post-euthanasia.

The intact animal model was chosen to mimic the clinical condition, where multiple factors interact to produce the final insult. In addition these infants have increased risks of other complications and thus the regional effect of the inotrope is an important factor before a conclusion is made. The newborn swine has been extensively used in perinatal and neonatal cardiovascular research. Newborn piglets are of similar size and degree of maturity to humans. Pigs are physiologically and anatomically similar to humans with similar size and distribution of blood vessels. The cardiovascular responses of the newborn piglet to stresses such as hypoxia are similar to those of human neonate.

In the current study, newborn piglets of 1-3 days old were subjected to alveolar hypoxia for 50 min and then resuscitated with 21% or 100% oxygen for 1 h and followed by 21% for 3.5 h. After 2 h of reoxygenation, animals were treated with either dopamine or epinephrine for low blood pressure and cardiogenic shock. *This is similar to the clinical practice, where inotrope infusion is initiated usually 1-3 h following asphyxia injury when the neonate develops hypotension, poor perfusion or oliguria.*

In this model, alveolar hypoxia was of adequate duration and severity ($SaO_2 \leq 35\%$) to induce significant shock (42% drop in cardiac index) and metabolic acidosis ($pH \leq 7.07$). We chose this level of asphyxia because the risk of morbidity and mortality is high in these infants compared to mild and moderate asphyxia. Although no common definition for the severity of asphyxia was found in the literature, cord arterial blood of $pH < 7.10$ confirms significant perinatal hypoxia. However there was no hypercapnia associated which may limit our finding to those asphyxiating events with hypoxia, normocapnia and metabolic acidosis. *Nonetheless our model is a practical and clinically related one for neonatal hypoxia-reoxygenation injury.*

To ensure that subjects in each treatment group are comparable in both known and unknown prognostic factors and that any differences in outcome in the treatment groups are due mostly to differences in treatment, the animals were blocked-randomized. The catecholamine infusion was prepared according to a written protocol by a research assistant independent from the primary investigator. The catecholamines treatments were administered in a double-blinded fashion by the primary investigator to avoid any bias. A double-blinded randomized controlled study that is seldom used in basic science experiments is the most rigorous clinical research design because, in addition to the randomization of subjects which reduces the risk of bias, it can eliminate the placebo effect which is a further challenge to the validity of a study.

Our results showed that post asphyxia, piglets reoxygenated with 100% oxygen had worse though not statistically significant cardiogenic shock when compared to piglets in the 21% reoxygenated group. In piglets resuscitated with either 21% or 100% oxygen, the low dose of either drug is not effective to improve systemic or renal perfusion. However epinephrine infusion (0.5-2 mcg/kg/min) is an effective inotrope for the treatment of shock in piglets resuscitated with 100% oxygen, and does not adversely affect regional perfusion. Indeed it has a positive effect on common carotid flow and at dose of 0.2 mcg/kg/min epinephrine improves superior mesenteric flow. While dopamine (10-25 mcg/kg/min) has a weak vasopressive action, it has a positive effect on mesenteric perfusion.

The treatment of epinephrine for 2.5 h is associated with increased oxidative stress in the left ventricle tissue of piglets resuscitated with 100% oxygen. Catecholamines are reported to have the dual nature of antioxidant and proxidant functions. It appears that the balance between the antioxidant and proxidant capacities of catecholamines is dictated by ambient conditions such as catecholamine concentration, ROS production. pH and oxygen content. This may at least explain in part the increase in degree of oxidative stress observed in the epinephrine group resuscitated with 100% oxygen following hypoxia. Furthermore the proxidant activity of a drug is thought to be directly proportional to the total number of free hydroxyl groups and as epinephrine contains more free hydroxyl groups than dopamine (3 and 2 respectively) this may explain the proxidant effect of epinephrine compared to dopamine.

Other limitations of this study are species differences, the hypoxia-reoxygenation protocol and the time of the catecholamines administration. Although we believe the experiment closely mimics the clinical scenario, generalization of our findings in the clinical practice requires great caution. For example clinically catecholamines treatment for hypotension and shock in the newborn is usually initiated if no response is observed to crystalloid fluid bolus. It can be given as early as 30 min after resuscitation as seen clinically and in some animals. Indeed in our pilot study the initiation of catecholamines at 3 h of reoxygenation was too late and most of the animals failed to respond to the treatment.

We believe that when 100% oxygen is used to resuscitate neonates with hypoxia, epinephrine compared to dopamine is more effective and should be the drug of choice for the treatment of shock and hypotension in these infants, without compromising the regional flow. Indeed it may increase carotid and mesenteric artery flows even at a low dose of 0.2 mcg/kg/min. Dopamine however has a systemic vasopressive action and may have a role to improve the mesenteric perfusion in asphyxiated infants, although it does not have significant positive inotropy. The use of low dose dopamine for the treatment of renal hypoperfusion associated with asphyxia may be futile as the effect of this treatment on the developing renal function of the newborn is still not clear and the effectiveness of this treatment in improving the renal perfusion in asphyxiated infants is not evidenced. The clinical significance of the increased oxidative stress observed with epinephrine treatment following 100% reoxygenation is not clear and needs further studies if this is related to the myocardial injury or subsequent dysfunction observed with prolonged administration.

Appendices

Appendix 1

Novel therapies in pulmonary hypertension:

Selective pulmonary vasodilators

Pulmonary hypertension (PHT) in the newborn can complicate any lung disease. It can occur in the presence of lung parenchymal disease (appropriate or secondary PHT) where alveolar oxygen tension appears to be the major determinant of pulmonary artery vasoconstriction. Pulmonary hypertension can complicate diseases that cause acidosis, hypoxia, and hypercapnia such as meconium aspiration syndrome, severe respiratory distress syndrome and asphyxia. It is a serious complication of neonatal asphyxia, in which the incidence of lung injury can occur up to 86% of infants exposed to hypoxia.[1]

Pulmonary hypertension may also occur with no evidence of parenchymal disease (persistent or primary PHT) where there is abnormal responsiveness of the pulmonary vasculature to hypoxia with an inability to relax after the stimulus for vasoconstriction is removed. The prevalence of pulmonary hypertension of the newborn is 1.9 per 1000 live births.[2]

The placenta is the organ of gas exchange in utero. The fetal lung is characterized by supra-systemic pulmonary arterial pressure and vascular resistance. This is needed to ensure adequate blood flow to the placenta. The transition to the postnatal life of low pulmonary arterial pressure requires vasodilation of pulmonary vessels and their structural remodeling. Disturbance of this process can lead to the clinical syndrome of persistence pulmonary hypertension.

Within the first 5 min after birth, there is greater than tenfold decrease in pulmonary vascular resistance along with an eight- to tenfold increase in pulmonary blood flow. The stimuli that seem to be most important in decreasing pulmonary vascular resistance and increasing pulmonary blood flow are ventilation of the lungs with a gas and an increase in oxygen tension.[3] Systemic vascular resistance also increases at birth, in part because of the removal of the low resistance bed of the placenta. Over the first few hours after birth, the ductus arteriosus closes, largely in response to the increase in blood oxygen tension. At this point, the normal postnatal circulatory pattern is established. This crucial transition at birth from fetal to postnatal circulation is influenced by an array of stimuli and vasoactive products.

The smooth transition from the high-resistance pulmonary vascular bed in utero to the low-resistance circuit after birth is mediated by vasoactive products released by the endothelium in the pulmonary vasculature at birth. These products include NO and arachidonic acid metabolites.[4] Nitric oxide is a potent dilator of the fetal and infant pulmonary circulation.[5] Nitric oxide activates soluble guanylate cyclase to produce cGMP. Cyclic guanosine monophosphate induces relaxation of smooth muscle in the perinatal pulmonary circulation via the activation of a cGMP-dependent protein kinase.[6] Oxygen is an important stimulus for endothelial production of NO during this transition although the mechanism is unclear.[7] Arachidonic acid is degraded by different enzymes to produce a variety of inflammatory mediators, including thromboxane, platelet-activating factor, leukotrienes C_4 and D_4 and prostaglandins. Thromboxane and leukotrienes are potent vasoconstrictors.[8] Prostaglandins are either vasodilators (prostacyclin) or vasoconstrictors ($PGF\alpha_2$). Prostacyclin is a potent pulmonary vasodilator that relaxes smooth muscles via the production of cAMP.[9]

The vasoactive response of the pulmonary vasculature is also mediated by oxygen-sensitive voltage-gated potassium channels (Kv); these are found in the smooth muscle of pulmonary artery cells in resistance arteries and they set the membrane potential of these vessels. Most of the understanding of the role of the K^+ in the pulmonary vasculature was obtained from studies investigating the hypoxic pulmonary vasoconstriction reflex; a response which is believed to be intrinsic to pulmonary artery smooth muscle.[10] Nine families of Kv channels are recognized from cloning studies (Kv1-Kv9), each with subtypes.[11] Hypoxia inhibits one or more Kv channels, resulting in membrane depolarization, opening of voltage-gated calcium channels, raising cytosolic calcium concentration and initiating constriction. One of the proposed mechanisms for the sensitivity of Kv to oxygen is that these channels respond to the local redox environment. Certain Kv channels are rich in cysteine, and tend to open when oxidized and close when reduced. Electron donors such as nicotinamide adenine dinucleotide phosphate oxidase, (NADPH oxidase) and the cytosolic ratio of reduced/oxidized redox couples (i.e. GSH/GSSG ratio) are controlled by electron flux in the mitochondrial electron transport chain. Changes in the ratio of reduced/oxidized redox couples, such as NADH/NAD and GSH/GSSG have been shown to reduce or oxidize the K^+ channels, resulting in alterations of pulmonary artery tone.[12]

Excessive vasoconstriction has been related to endothelial dysfunction, leading to reduced production of vasodilators such as NO and prostacyclin along with overexpression of vasoconstrictors such as endothelin,[13] and abnormal function, inhibition or expression of potassium channels. There is also the release of inflammatory substances, such as leukotriene C_4 and D_4, thromboxane and platelet-activating factor. These factors have been found to be elevated in infants with persistent pulmonary hypertension.[14] They are known to increase pulmonary arterial pressure and vascular resistance in newborn animals.[13] Hypoxia is a potent vasoconstrictor, especially below partial pressure of oxygen of 40-45 mmHg. Fetal partial pressure of oxygen averages 20-25 mmHg producing persistent active pulmonary vasoconstriction in utero.

Alkalinization, hypocapnia, sedation and vasodilators have been used for the treatment of PHT in the newborn when the infant fails to respond to increased inspiratory oxygen concentration and treatment of the underlying disease. Vasodilators such as tolazoline, prostacyclin and recently phosphodiesterase inhibitors such as sildenafil have been used for PHT treatment in the newborn. These agents however are not pulmonary selective and oral or intravenous administrations of these agents frequently result in vasodilatation of both the pulmonary and systemic vascular beds.

Endotracheal administration of tolazoline and aerosol administration of prostacyclin has been reported to be effective.[15,16] But the most widely used inhaled medication for the treatment of PHT is NO. This gaseous free-radical compound was previously known as endothelial derived relaxation factor. Inhaled NO directly activates soluble guanylate cyclase leading to increased levels of cGMP in vascular smooth muscle cells. This results in vascular relaxation by prohibiting myosin protein cross-bridge formation in smooth muscle. Unfortunately problems such as rebound PHT which may occur upon discontinuation, the need for a special delivery device, trained personals and the rising cost of this therapy may limit its use clinically.[17] Clinically oral phosphodiesterase inhibitors have been used successfully for the treatment of PHT associated with congenital heart disease and rebound PHT. Reports from adult animal studies indicate that the use of aerosol sildenafil may also be effective.[18] Furthermore PHT can occur in conditions where surfactant is depleted or inactivated and there is some evidence that surfactant administration may increase pulmonary blood flow and decrease pulmonary vascular resistance.[19]

In summary, PHT is a serious complication which may occur in the absence of lung parenchymal disease in the newborn. The use of selective pulmonary vasodilators such as inhaled NO in the management of these infants avoids many of the systemic side effects which occur with oral and intravenous administration of vasodilating agents such as tolazoline. Technical difficulties, rebound PHT and the increasing cost of NO may limit it use and increase the need for novel treatment of PHT.

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Appendix 2

Intratracheal administration of sildenafil and surfactant alleviates pulmonary hypertension in the newborn pigs:

Novel selective pulmonary vasodilator treatment for pulmonary hypertension in neonates.

Introduction

In a variety of neonatal respiratory disorders including asphyxia, meconium aspiration syndrome, hyaline membrane disease and congenital diaphragmatic hernia, surfactant has been used effectively to improve oxygenation in hypoxemic respiratory failure.[1,2] Pulmonary hypertension (PHT) however can also complicate many of these conditions.[3,4] Administration of surfactant is also associated with changes in pulmonary arterial pressure.[5]

Nitric oxide (NO) when given by inhalation causes selective pulmonary vasodilatation and has been shown to be an effective therapy for term and near term newborns with PHT and respiratory failure.[6] Nitric oxide produces smooth muscle relaxation by increasing the intracellular level of cyclic guanosine monophosphate (cGMP).[7] However, there are potential disadvantages to its use: the danger of PHT rebound upon discontinuation,[8] concerns about toxicity at high dosage, enhancement of inflammation and alteration of homeostasis, and recently, its cost.[9] Alternative pulmonary vasodilating therapies are being studied.

Phosphodiesterase type V (PDE5) is found in high concentrations in the lung and contributes to cGMP degradation within vascular smooth muscle.[10] Sildenafil citrate, a selective phosphodiesterase type V inhibitor produces pulmonary vasodilatation when given through systemic or inhalation routes.[11,12] Ichinose et al demonstrated that, in an adult sheep model of acute PHT, aerosolized Sildenafil dose-dependently (1-30 mg) alleviated the PHT induced by a thromboxane A_2 analog (U46619, 1-6 mcg/kg/min). The elevated pulmonary artery pressure (PAP) was reduced significantly (21-26 %) with 10-30 mg (0.4-2 mg/kg) of sildenafil, which was not associated with any impairment of systemic oxygenation. Aerosolized sildenafil however did not significantly prolonged the duration of pulmonary vasodilatation produced by inhaled NO. No significant changes on mean arterial pressure (MAP), systemic vascular resistance or cardiac output were found, as measured in response to the inhalation of ≤30 mg of aerosolized sildenafil.[12]

Because of the physical property of surfactant,[13,14] we attempted to use surfactant as the vehicle to deliver Sildenafil such that a low dose can be used to achieve the therapeutic effect without significant systemic effects. The hemodynamic effects of surfactant and low-dose Sildenafil administered in combination on the systemic and pulmonary circulations have not been examined. We therefore designed the following experiment to test the hypothesis that the use of intratracheal low-dose Sildenafil (0.05 mg/kg) with surfactant is effective in alleviating PHT in newborn piglets with minimal systemic hemodynamic effects.

Methods

Animals

Duroc piglets (1–3 days of age, weighing 1.7-2.5 kg) were obtained from a local farm on the day of experiment. The study conformed to the regulation of the Canadian Council of Animal Care and was approved by the Health Science Animal Welfare Committee, University of Alberta.

Anesthesia

Anesthesia was induced with inhaled halothane 5% and maintained at 2%. Following tracheotomy (size 3.0 endotracheal tube, Mallinckrodt Critical Care, Glenn Falls, NY) and commencement of mechanical ventilation, halothane was discontinued and anesthesia was maintained with intravenous infusions of Fentanyl 5-15 mcg/kg/h, Midazolam 0.1-0.2 mg/kg/h and Pancuronium 0.05-0.1 mg/kg/h. Boluses of these drugs were given if needed.

Surgical procedure

A 5-French Argyle™ single lumen arterial catheter (Sherwood Medical Co., St. Louis, MO) was inserted 5cm into the right common carotid artery and was attached to a pressure transducer for the continuous monitoring of MAP and heart rate (HR). A 5-French Argyle™ double lumen catheter was inserted 5cm to the level of right atrium via right external jugular vein for the administration of fluids and central venous pressure measurement. Dextrose-saline solution was infused at a rate of 10 ml/kg/h. Piglets were ventilated (Sechrist infant ventilator model IV-100, Sechrist Industries Inc., Anaheim, CA) at pressures of 16/4 cm H_2O at a rate of 12-18 breaths per min with inspired oxygen concentration (F_iO_2) of 21-30%. The F_iO_2 was measured by an Ohmeda 5100 oxygen monitor (Ohmeda Medical, Laurel, MD) and adjusted accordingly to maintain oxygen saturation at 89-95%. Further intravenous doses of Fentanyl (10 mcg/kg) and Pancuronium (0.1 mg/kg) were given prior to a left anterior thoracotomy. The pericardium was opened and a 20-gauge Insyte-W™ (Becton Dickinson Infusion Therapy Systems Inc., Sandy, UT) was inserted into the main pulmonary artery and attached to a pressure transducer to continuously measure PAP. A six-millimetre transit time ultrasound flow probe (6SB906, Transonic Systems Inc., Ithaca, NY) was placed around the main pulmonary artery to measure cardiac output. The surgical wounds were covered with wet gauze.

Experimental protocol

Following the completion of surgical instrumentation, blood gases were drawn and 15 min of recording was done to ensure the animal was stable. Stability was defined as (1) HR and hemodynamic parameters within 10% of the post-anaesthetic prethoracotomy values, (2) Central venous pressure of 3-8mmHg (3) P_aCO_2 7.35-7.45. The surgical procedure usually finished within 35 min. The rectal temperature was maintained between 38.5-39.5°C by an electrical heating blanket and an infrared-heating lamp.

After a baseline monitoring period of at least 30 min, simultaneous blood samples were drawn for arterial, mixed venous oxygen saturation and hemoglobin concentration determinations by co-oximeter (OSM2 Hemoximeter, Radiometer, and Copenhagen). Arterial blood gases were also performed (ABL500, Radiometer). The following hemodynamic variables were monitored continuously throughout the study period: HR, MAP, PAP, central venous pressure, pulmonary artery flow (cardiac output), and transcutaneous oxygen saturations by a pulse oximeter (Nellcor, Hayward, CA). Analogue outputs of the pressure amplifiers and flow monitors were digitized by a DT 2801-A analogue to digital converter board (Data Translation, ON) in a Dell 425E personal computer. Software was custom written using the Asyst programming environment. All signals were continuously acquired at 24 Hz and saved on hard disk. These variables were monitored continuously for 2 h after stabilization.

Pulmonary hypertension was induced by U46619 infusion (0.2-0.8 mcg/kg/min) in order to produce at least a 50% increase in PAP above baseline with the F_iO_2 increased to 40%. Thereafter the dose of U46619 was freshly maintained at the same rate throughout the remaining period of the experiment. After 60 min of U46619 administration, piglets were blindly randomized for intratracheal administration of surfactant (BLES, 4 ml/kg) with saline (1 ml) (control, n=6) or Sildenafil (0.05 mg/kg dissolved in 1 ml of saline, pH 4) (n=6). Sildenafil (Pfizer Inc., Canada.) was prepared fresh prior to the experiment. The surfactant with either Sildenafil (0.05 mg/kg) or normal saline was given slowly over 5 min with the F_iO_2 increased to 1.0. The F_iO_2 was then gradually reduced to 40% within 15 min and remained at 40% for the remaining period of experiment. The hemodynamic variables at 0, 30, 60, 90 and 120 min of U46619 infusion were recorded over 2 min for analysis. Simultaneous oxygen saturation of arterial (S_aO_2) and mixed venous (S_vO_2) blood were also measured at these time points. These variables were collected by one of the authors (LO) with no knowledge of the animal randomization. No bicarbonate solution was given during the experiment.

Formulae

Cardiac index (CI), systemic vascular resistance, stroke volume index, systemic oxygen delivery, oxygen consumption and oxygen extraction ratio were calculated as follows:

CI = Pulmonary artery flow ÷ body weight

Stroke volume index = CI ÷ heart rate

Systemic vascular resistance index = (MAP – Central venous pressure) ÷ CI

Systemic oxygen delivery = CI x Hb x 1.34 x S_aO_2

Systemic oxygen consumption = CI x Hb x 1.34 x ($S_aO_2 - S_vO_2$)

Systemic oxygen extraction ratio = ($S_aO_2 - S_vO_2$) ÷ S_aO_2

The pulmonary vascular resistance index (PVRI) was estimated by dividing the PAP with CI.

The PAP: MAP ratio, an indicator of the relative selectivity of the vascular action on pulmonary and systemic circulation was also calculated.

At the end of the experiment the animals were euthanized with an intravenous overdose of pentobarbital (240 mg).

Statistical analysis

Data are expressed in mean±SEM and were analyzed by two-way repeated measures analysis of variance (RM ANOVA) using a statistical software (Sigma Stat 1.01 version, Jandel Scientific, San Rafael, CA). Dunnett's post-hoc test was used to compare the difference with baseline. A P value of <0.05 was considered significant.

Results

Twelve piglets (weight 2.0±0.04 kg) were instrumented and studied. The baseline physiological characteristics are shown in (Table A2-1).

U46619 treatment caused progressive PHT (PAP: 161 and 152 % of respective baselines of control and study groups; PVRI: 290 and 208 % of respective baselines of control and study groups; respectively). Surfactant alone did not alleviate the increased PAP and PVRI (Fig A2-1 and A2-2). Surfactant and low-dose Sildenafil combined decreased PAP (33±5 vs. 41±2 mmHg of controls, $P<0.001$) along with a significant reduction in PVRI (0.25±0.03 vs. 0.42±0.06 mmHg.kg.min/ml of controls, $P<0.001$) (Fig A2-1 and A2-2). This reduction in PAP and PVRI amounted to 11% and 7% of study group after 60 min of U46619 infusion, respectively.

With the infusion of U46619, CI decreased significantly from the baseline (57%). At 120 min of U46619 infusion, the treatment with surfactant and Sildenafil combined produced a higher CI than that of controls (145±15 vs. 108±14 ml/kg/min, respectively, $P<0.05$). The MAP did not change significantly during the infusion of U46619. There was no significant effect on the MAP with surfactant and Sildenafil combined compared to that with surfactant alone (76±4 and 78±6 mmHg, respectively).The PAP: MAP ratio was increased with U46619 treatment (158%) and the increase was significantly reduced by surfactant and Sildenafil but not surfactant alone (0.39±0.06 vs. 0.51±0.06, respectively, $P<0.001$) (Fig A2-3).

No significant differences were observed in heart rate (182±8 and 204±14 bpm) and stroke volume index (0.69±0.09 and 0.55±0.08 ml/kg) between the study and control groups, respectively, and also when compared to the respective baseline values. Systemic vascular resistance index (0.59±0.07, 0.75±0.1 mmHg.min.kg/ml) was not different between the groups at 120 min of U46619 administration.

Systemic oxygen delivery was significantly reduced during U46619 infusion but was not different between the groups (Table A2-2). No significant effect on the systemic oxygen consumption was observed during the U46619 infusion and between the groups. Regarding the systemic oxygen extraction ratio, a significantly increase was noted in the group of piglets receiving surfactant alone compared to that of piglets treated with surfactant and Sildenafil at 120 min, which was not different from the baseline value (Table A2-2).

Discussion

This study is the first to demonstrate the effectiveness of low-dose Sildenafil combined with surfactant on alleviating PHT in newborn piglets. The reduction of PAP and PVRI by 11% and 7% may be clinically important. The addition of Sildenafil to surfactant has the potential as a novel combination in the treatment of newborns and infants at risk for the development of PHT when surfactant is warranted.

In a pilot study, we attempted to study the effect of sildenafil and surfactant in PHT induced by alveolar hypoxia, but we were faced with high mortality with the administration of surfactant under reduced FiO_2. In the current study, we examined the effect of adding low-dose Sildenafil (0.05 mg/kg) to intratracheal instillation of surfactant in a porcine model of neonatal PHT secondary to thromboxane infusion. In this model, we used U46619, a thromboxane A_2 analog, to induce PHT in the neonatal piglets. Thromboxane mediates PHT and an increase of plasma thromboxane A_2 release occurs in both the primary and secondary forms of PHT.[15] The use of thromboxane A_2 analog U46619 in inducing PHT hypertension in animal models has been described previously.[12]

Recently Sildenafil has been advocated as an adjunctive therapy for neonates with PHT in part related to its mechanism of vascular action via the cGMP pathway similar to that of inhaled NO. Sildenafil causes vasodilatation by enhancing and prolonging the action of cGMP via the selective inhibition of the cGMP-specific PDE5 isozyme.[16] Because of a predominant expression of PDE5 in the lung vascular tissue,[17] Sildenafil provides a selective pulmonary vasodilating effect. Indeed, the selective PDE5 inhibition by Sildenafil may cause a longer lasting reduction in mean PAP and PVRI, compared to other nonselective PDEs inhibitors.[11]

Oral and intravenous Sildenafil reparations are reported to be as effective as inhaled NO in causing pulmonary vasodilatation,[18,19,20 ,21 ,22 ,23] with a combination of the two being more effective than either therapy alone. Sildenafil was also found to attenuate the rebound PHT associated with withdrawal of inhaled NO (11). Sildenafil can potentiate the effects of inhaled NO when given orally, as an intravenous infusion or in an aerosolized.[12,24,25] Interestingly, Ichinose et al demonstrated that aerosols of 10-30 mg of Sildenafil (0.4- 2 mg/kg) decreased PAP by 21±3% and 26±3%, respectively, which correlated with the plasma Sildenafil concentrations.[12]

Previous studies reported that Sildenafil given intravenously, the most common route of administration of medications in critically ill newborns had no effect on systemic arterial pressure. Shekerdemian et al examined the effects of both inhaled NO and intravenous Sildenafil in a porcine model of neonatal PHT secondary to meconium aspiration.[25]. Intravenous Sildenafil reversed PHT with lowering of mean aortic pressure up to 11%. Furthermore, Schulze and Redington[26] raised concerns with the use of systemic administration of Sildenafil regarding systemic oxygenation. Although the systemic administration of Sildenafil is “relatively” selective for the pulmonary circulation, it is not selective in terms of its effects within that circulation. Unlike inhaled NO, where its site of action is restricted to the ventilated areas of lung,[27] systemic Sildenafil can potentially reverse hypoxic vasoconstriction (the Euler-Liljestrand reflex) secondary to alveolar hypoxia and lead to increased ventilation-perfusion mismatch and thus systemic hypoxemia. Schulze et al showed a fall in arterial P_aO_2 of an average of 10-15 mmHg in children with PHT after cardiopulmonary bypass surgery treated with intravenous sildenafil.[28]

Shekerdemian et al also examined the co-administration of intravenous Sildenafil (2 mg/kg) and inhaled NO in a porcine model of PHT [29] and observed serious adverse side effects of systemic vasodilation with hypotension and deterioration of oxygenation. It was not clear whether these unwanted effects were caused, at least in part, by an inappropriately high dose of sildenafil and due to a redirection of blood flow away from ventilated areas toward inappropriately vasodilated but unventilated areas of the lung. While the optimal Sildenafil dose and mode of administration in the pediatric population are not known,[16,29] the use of an intratracheal low-dose of highly selective PDEs-5 inhibitor with surfactant might minimize the effect of the pulmonary and systemic side effects. Indeed, in PHT associated with acute parenchymal lung injury, such as meconium aspiration syndrome, the mode of delivery of the vasoactive agent may play a crucial role in providing the optimal pulmonary vasodilation without systemic side effects. Here we showed that surfactant and low-dose Sildenafil (0.05 mg/kg) combined significantly alleviated PHT while surfactant alone did not alleviate the increased PAP and PVRI, and systemic oxygen delivery was not impaired. By adding Sildenafil to surfactant, the dose can be reduced because surfactant acts as the vehicle to improve the delivery to the parenchymal vascular sites. In a pilot study, we did not observe any significant effect on PAP and PVRI with the administration of Sildenafil (0.05 mg/kg) alone. This is consistent with the observation of Ichinose et al using 1 mg of aerosolized Sildenafil in 12.5 -25 kg lambs with PHT. It also has the theoretical advantage in that by providing selective vasodilatation in the ventilated area of the lung without increasing ventilation-perfusion mismatch thus may improve systemic oxygenation.

Exogenous surfactant application is an effective treatment option in neonates with hyaline membrane disease and meconium aspiration syndrome. Surfactant improves gas exchange and decreases the use of ventilatory support.[1,2] Pulmonary hypertension is commonly associated with these lung diseases. We propose that the addition of low-dose Sildenafil to surfactant may have a role in the treatment of PHT associated with these conditions with minimum systemic adverse effects. Indeed, an increase in PAP may occur when surfactant is used in these situations and this may aggravate the PHT and systemic oxygenation. Our study is limited by the experimental protocol that it was not originally designed to study the effect of surfactant and Sildenafil combined in the animal model of these pathological conditions. Extrapolating the findings in this study to the above diseases requires caution and needs further research.

In conclusion the addition of low-dose Sildenafil to surfactant is effective in alleviating thromboxane A_2 induced PHT in newborn piglets. Further research is required to examine the potential the combination of Sildenafil and surfactant as a novel therapeutic adjunct in the management of critically ill neonates.

Table A2-1: Physiological characteristics

Physiological characteristics after one hr of U46619 infusion of 12 piglets treated with surfactant and saline (control, n=6) or Sildenafil (study, n=6). Mean±SEM

	Baseline		1h of U46619	
	Control	Study	Control	Study
pH	7.32±0.02	7.35±0.02	7.28±0.02	7.27±0.02
PCO_2	42±2	38±1	40±3	43±2
PO_2	67±7	72±3	151±20	151±20
Heart rate (bpm)	209±16	196±10	181±18	173±13
Cardiac index (ml/kg/min)	214±17	204±14	123±16	156±11
Stroke volume index (ml/kg)	1.04±0.08	1.01±0.09	0.72±0.11	0.82±0.13
Mean arterial pressure (MAP) (mmHg)	70±5	70±2	77±2	81±3
Pulmonary artery pressure (PAP) (mmHg)	22±1	24± 2	36±2	37±4
Systemic vascular resistance index (mmHg.min.kg/ml)	0.31±0.04	0.33±0.03	0.63±0.06	0.60±0.08
Pulmonary vascular resistance index (mmHg.min.kg/ml)	0.11±0.01	0.13±0.02	0.32±0.04	0.27±0.04
PAP/MAP ratio	0.27±0.03	0.28±0.04	0.44±0.04	0.44±0.04

Values are means±SEM

Table A2-2: Systemic oxygen metabolism

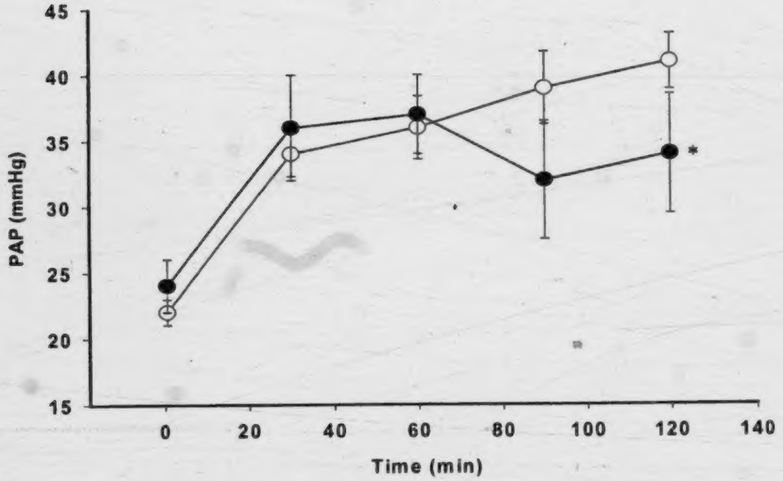
Systemic oxygen delivery (DO_2), consumption (VO_2) and extraction (EO_2) of 12 piglets treated with surfactant and saline (control, n=6) or Sildenafil (study, n=6) at baseline and at 120 min after U46619 infusion.

	Baseline		120 min of U46619	
	Control	Study	Control	Study
$SysDO_2$ (mL/kg min)	20±3	19±1	11±2	13±1
SysVO2 (mL/kg min)	9±1	8±1	6±1	5±1
$SysEO_2$	0.38±0.06	0.36±0.04	0.49±0.06*†	0.33±0.02

* P<0.05 vs. control, vs. Baseline, † (ANOVA). Values are means±SEM

Fig A2-1: Pulmonary arterial pressure

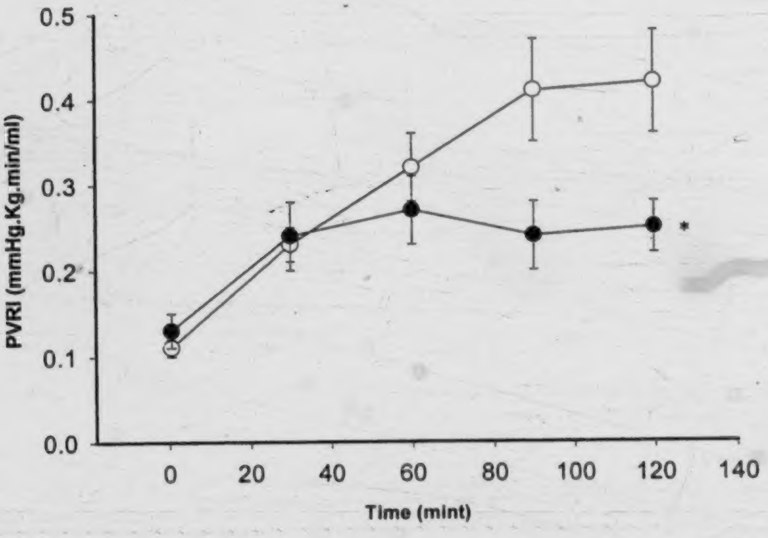
Pulmonary hypertension was induced by U46619 infusion (0.2-0.8 mcg/kg/min) in order to produce at least 50% increase in PAP above baseline with the F_iO_2 increased to 40%. Thereafter the dose of U46619 was maintained at the same throughout the remaining period of the experiment. After 60 min of U46619 administration, piglets were blindly randomized for intratracheal administration of surfactant (BLES, 4 ml/kg) with saline (1 ml, control (o)) or Sildenafil (0.05 mg/kg dissolved in 1 ml of saline (•)).



* P< 0.05 vs. control. Values are means±SEM.

Fig A2-2: Pulmonary vascular resistance

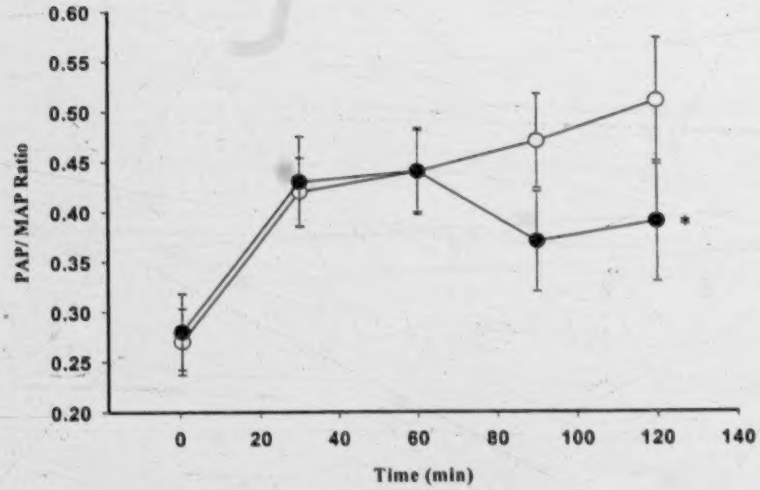
Pulmonary vascular resistance (PVRI) at baseline and during U46619 infusion (0.2-0.8 mcg/kg/min). The U46619 infusion was increased in order to produce at least 50% increase in PAP above baseline with the F_iO_2 increased to 40%. Thereafter the dose of U46619 was maintained at the same throughout the remaining period of the experiment. After 60 min of U46619 administration, piglets were blindly randomized for intratracheal administration of surfactant (BLES, 4 ml/kg) with saline (1 ml, control (o)) or Sildenafil (0.05 mg/kg dissolved in 1 ml of saline (•)).



*P< 0.05 vs. control. Values are means±SEM

Fig A2-3:PAP/MAP ratio

Pulmonary to systemic arterial pressure (PAP/SAP) at baseline and during U46619 infusion (0.2-0.8 mcg/kg/min). The U46619 infusion was increased in order to produce at least 50% increase in PAP above baseline with the F_iO_2 increased to 40%. Thereafter the dose of U46619 was maintained at the same throughout the remaining period of the experiment. After 60 min of U46619 administration, piglets were blindly randomized for intratracheal administration of surfactant (BLES, 4 ml/kg) with saline (1 ml, control (o)) or Sildenafil (0.05 mg/kg dissolved in 1 ml of saline (•)).



*P< 0.05 vs. control. Values are means±SEM

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Appendix 3

Definitions

Hypoxia is a lack of an adequate supply of oxygen to the tissues despite adequate perfusion, leading to a deficiency in the amount of oxygen reaching body tissues.

Ischemia is a decrease in the blood supply to a bodily organ or tissue.

Asphyxia is hypoxia in the presence of acidosis and hypercapnia. It can lead to loss of consciousness or death.

Catecholamines are a group of chemical substances, which have various roles in the functioning of the sympathetic and central nervous system. Chemically, all contain a benzene ring with adjacent hydroxyl groups and an amine group on a side chain. The principle amines are norepinephrine, epinephrine and dopamine. All of these chemicals act directly or indirectly on the sympathetic nervous system, but the effect of each agent varies according to which adrenergic and dopaminergic receptors the drug has greatest affinity for. The duration of action also varies. Direct acting drugs (e.g. epinephrine) act by stimulating the sympathetic nervous system receptor, whereas indirect acting drugs (e.g. dopamine) cause the release of norepinephrine from the receptor that produces the effect. Some drugs have a mixed effect.

Monoamine is a chemical substance contains one amino acid.

Vasopressors are a powerful class of drugs that induce vasoconstriction and thereby elevate systemic arterial pressure.

Inotropes are pharmacologic agents that affect the rate of contraction of myocardial fibers. Positive inotropes increase and negative inotropes decrease the myocardial contractility. Many drugs have both vasopressor and inotropic effects. This group of drugs is useful for resuscitation of seriously ill patients, and for the treatment of hypotension.

Vasoactive drug is a term that can be used for either an inotrope or a vasopressor. All vasoactive drugs are short acting and are given by infusion into a central vein.

Chronotropy, changes in heart rate.

Inotropy, changes in heart contractility.

Dromotropy, changes in conduction velocity.

Shock is a clinical syndrome of circulatory dysfunction resulting in inadequate oxygen and nutrient delivery, with inability to meet the metabolic demands of the cells. This results in a cascade of events resulting in altered cellular metabolism, function, structure, and ultimately death. Shock is not necessarily associated with hypotension. It begins with a normal blood pressure and progresses over time.

Hypotension refers to a blood pressure that is lower than the expected reference range. Although the normal physiologic range for the blood pressure is not well studied in the newborn population, in clinical practice, the reference range blood pressure limits are defined as the gestational and postnatal age–dependent blood pressure values between the fifth (or 10th) and 95th (or 90th) percentiles.

Reduction/Oxidation or redox; reduction is the act of neutralizing a free radical (adding an electron) or adding a hydrogen atom to a compound to reduce double bonds between atoms. Oxidation is the act of removing an electron from a stable orbital or reducing the number of single bonds and increasing the number of double bonds. This is usually done by either removing hydrogen or by adding oxygen sometimes both, thus the name oxidation. Redox potential is the ease of being oxidized or reduced (simply put).